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EXSHEATHING AND STERILIZING INFECTIVE NEMATODE LARVAE

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To obtain easily and consistently the second ecdysis of strongyloid nematode larvae (thus releasing the third larval, i.e. the first parasitic stages) and secure them in quantity under sterile conditions, is a necessary aim of workers interested in the artificial culture of these forms. The second molt "skin" developed at the end of free-living existence remains as a resistant coat which in many species subserves a protective function by preventing dehydration and other deleterious effects of the environment. Normally this cuticle is shed at the time or after the parasite enters its host. While infective nematodes can be caused to migrate through ground glass and carborundum powder to tear the second molt cuticular covering from the third stage larva within, such methods are cumbersome and produce inconstant results. Moreover, when by these methods exsheathed first parasitic forms have been obtained, they are not sterile in the bacteriological sense and culture work with them is thus not possible.

Lapage was the first worker to develop an easy and practical method whereby the second ecdysis of large numbers of nematode larvae could be secured. This worker was able to obtain the species used under sterile conditions, so that the first parasitic stages could be put into any desired sterile medium, although he failed to get any further ecdysis or development in the many different media which he used.

In 1933 Lapage reported (1) that the infective stages of *Graphidium strigosum* and *Trichostrongylus retortaeformis,* nematodes of the rabbit, could be freed from their second stage sheaths by placing them in solutions containing hypochlorite, of which he found "Milton" to be best. In 1 part of Milton solution to 20 parts of distilled water, the larvae exsheathed in 20 minutes or less at laboratory temperatures. Stronger dilutions damaged or killed them. Lapage favored Milton because it remains stable and exsheathed larvae will live 3 or 4 days in the dilution of 1 to 20.

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In 1935 Lapage (2) extended these important observations to *Haemonchus contortus, Ostertagia circumcincta,* and species of *Trichostrongylus,* nematodes parasitic in sheep. A number of different chemicals were tried successfully for the artificial production of ecdysis, but none were found as useful as Milton hypochlorite in respect to both exsheathing and sterilizing effects.

In another paper Lapage (3) more fully described the experimentally produced second ecdysis. The larva is passively ejected part of the way out through a hole at the anterior tip of the sheath and completes its emergence by its own movements. Lapage states: "Milton solution alters the chemical composition of the sheath with two results: (a) the permeability of the sheath is altered so that increased internal pressure results, by which the larva is propelled out of the hole at the anterior tip, and (b) the sheath is rendered soluble in the NaOH in the Milton solution so that it is subsequently dissolved. The larva is expelled through a hole at the anterior tip before the whole sheath is dissolved because the sheath gives way here first to the combined effects of the increased internal pressure and the solvent action of the NaOH, this being its weakest point, where the remains of the mouth of the second larva are." He concludes by stating: "Further investigations of the second ecdyses by biophysical methods may bring all the ecdyses of parasitic nematodes under experimental control and so make possible the artificial cultivation of the adult parasites."

EXPERIMENTAL

In our attempts to cultivate the parasitic stages of certain sheep nematodes (4) it became imperative to obtain quantities of exsheathed and sterile third stage forms. At first we were unable to obtain Milton solution, so decided to use a similar, easily obtained product known as "Labarraque's solution" containing not less than 2.6 per cent of sodium hypochlorite in contradistinction to 1.01 per cent in Milton. Like the latter preparation, Labarraque's solution is stated to be a deodorizer, preservative, insecticide, and general antiseptic. It is said not to be so stable as Milton solution and should be stored in well-stoppered bottles, in a cool place, protected from light. A 1 to 20 dilution of Labarraque's solution readily caused the exsheathing of the infective stages of *Haemonchus contortus, Ostertagia circumcincta,* and *Trichostrongylus axei* of sheep; *Obeliscoides cuniculi* of rabbits; and *Nippostrongylus muris* of the rat. Presumably it will act similarly on strongyloid forms generally.

The details of the process of exsheathing in Labarraque's solution compare in general with those described by Lapage for Milton solution. However, we do not believe that the movements of the larva are essential in completing its emergence from the sheath. When the mature free-living larvae of *Haemonchus contortus* are rapidly killed in hot water

and then placed in the Labarraque dilution of 1 to 20, the dead, rigid forms are ejected from their sheaths as readily as the living larvae. We therefore think that the solvent action of Labarraque on the sheath and the increased intrasheath pressure produced are probably the main factors concerned. The fact that the living larvae lash violently when the anterior tip gives way, no doubt assists the ejection materially, but the movements are not essential. The chief factors appear to be physico-chemical.

In Fig. 1 are illustrated the infective larvae of *H. contortus,* and in

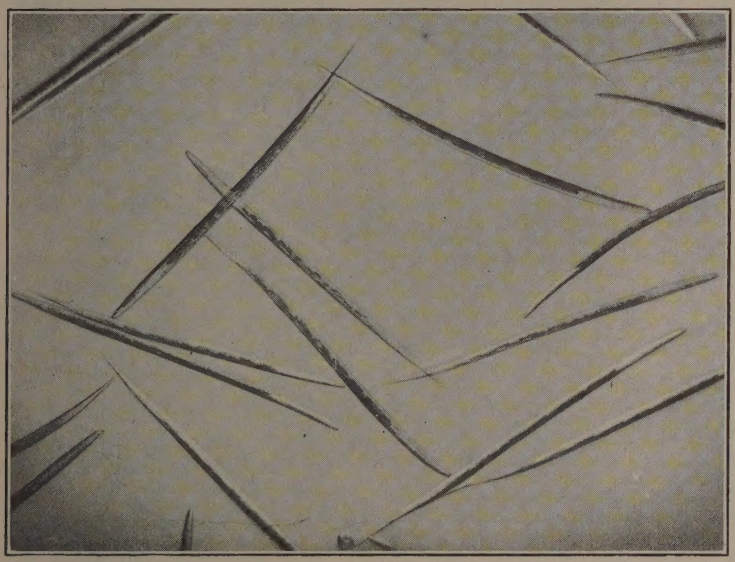

FIG. 1. Infective *Haemonchus contortus* larvae in their end-2nd stage sheaths. Heat-killed. ×90. (Photograph by J. A. Carlile.)

Fig. 2 the exsheathed forms after Labarraque treatment. The wrinkled and somewhat telescoped appearance of the cast sheaths is characteristic. There is subsequent solution and disappearance of the sheaths during exposure to the hypochlorite.

While exsheathing was thus readily accomplished, in our hands and with our methods, it has not been so simple to surely *sterilize* the larvae of *Haemonchus contortus, Ostertagia circumcincta,* and *Trichostrongylus axei.* Not infrequently bacteriological tests with routine laboratory media even after a week's incubation seemed to show that the larvae were sterile. However, when such larvae were placed in our enriched and partially anaerobic media used for nematode culture, bacteria or fungi often appeared, sometimes even after an incubation of two or three weeks.

Contaminated culture tubes are of no value, since parasitic stages of the nematodes here discussed only showed development in the absence of living microörganisms.

More effective sterilization resulted, however, when we lengthened the washing and sedimenting stages to be described. This lengthened period evidently allowed more of the microörganisms contained within the larvae themselves to be expelled, presumably by normal peristalsis. Once free, the bacteria were then either lost during the washing proce-

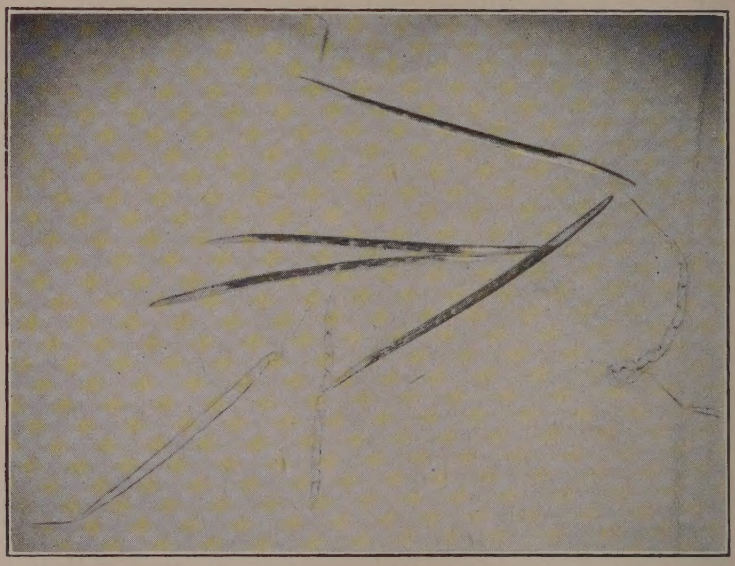

FIG. 2. Infective *Haemonchus contortus* larvae (heat-killed) which have been exsheathed in Labarraque's solution. The attenuated tail of the 2nd stage larvae shows in the cast sheaths, in contrast to the more stubby posterior tip of the freed 3rd stage forms. ×90. (Photograph by J. A. Carlile.)

dures, or were rendered available to chemical attack by the hypochlorite.

The following method, slightly modified from time to time, has yielded sterile larvae with very few exceptions during the past two years. The mature free-living infective larvae in feces are isolated in a "Baermann" funnel after which they are settled four times in 15 cc of sterile water. During this procedure a considerable amount of clumping may occur. The larger clumps as they settle together with coarse débris must be removed with a fine pipette and discarded. The nematode suspension should never be too dense; otherwise the occurrence of clumping adds to the danger of carrying along an excessive amount of contamination. Later on, should the nematode suspension be of the proper density for future work, and should a small degree of aggregation still occur, the clumps must be broken by sucking them in and out of a fine pipette.

The nemas are now permitted to settle in a sterile, long, glass tube held in a vertical position, containing about 15 cc of sterile water.* The nematodes gradually settle during the course of approximately one hour into the tapering end of the tube. The end of the tube is then inserted into 95 per cent alcohol for a few minutes. At the same time a file is inserted into the alcohol and flamed. The long tube is cut at the constriction with the sterilized file and the nemas transferred, with a small amount of the water, from the tapering end to a small sterile test tube (agglutination tube) by means of a sterile, finely drawn pipette. When the nemas have settled, the small amount of water is drawn off and about 5 cc of Labarraque's solution (or Milton's), diluted in the proportion of 1 part to 20 parts of water, introduced. After the nemas have again settled, the solution is drawn off and 5 cc of a fresh supply of Labarraque's solution added. This second addition of Labarraque is made because the first lot is still diluted beyond the 1 to 20 dilution by some original water associated with the larvae. In this second lot of Labarraque the nemas remain for ½ hour. During this time they become exsheathed and superficially sterilized.

At the end of this period the Labarraque is drawn off and the larvae are again settled in one of the long tubes. After settling and after the tube and file have been treated as previously described, the larvae are removed aseptically from the tapering tip and transferred to a tube containing 10 cc of sterile water. Here the parasites remain for 48 hours with two changes of sterile water during this time. This prolonged sojourn in water permits the larvae to digest and excrete viable bacteria within their alimentary tracts. Cultures taken from larvae at the end of the first day, i.e., up to the 48 hours' sojourn in water, often prove sterile when routine media are used. However, much time, energy, and material are saved if the procedure is properly concluded. As stated previously, special enriched media are used for our nematode culture work and these media often in time reveal the growth of microörganisms not shown by the routine methods. However, sterile cultures obtained at the end of this cleansing period signify that the nemas have been freed of much of their flora.

At the end of the 48-hour period the larvae are again settled in sterile water in one of the long tubes. They are then removed and placed in a 1 to 20 dilution of Labarraque freshly prepared. This is removed after the larvae have settled and fresh Labarraque added. Here the nemas

* This tube, first used in 1930 (5) for purifying protozoa, has proved very helpful in work with nematodes. The tube is about 14 inches long with a ¼ inch bore. The upper end is plugged with cotton and the lower end has a coarsely tapering point, 2 to 2½ inches long, having a slight constriction where the taper starts. The end of the taper is sealed in a flame after the tube is filled with sterile water.

Agitating the nema suspension by rotating the tube vigorously at intervals between the palms of the hands facilitates the settling.

are subjected to a further sterilization for ½ hour. The Labarraque is then drawn off and the larvae are again settled in a long tube. After removal from the long tube, in the customary manner, they are transferred three times to three separate lots (5 cc) of sterile Ringer's or Tyrode's solution of one-fourth concentration. From the last tube the nemas may be transferred to any desired medium with the assurance that the culture will remain sterile.

The above procedure is now standard in our laboratory and has been successfully performed as a routine by a laboratory technician for over a year. Other duties can be performed while the sterilization work progresses, but one must be careful not to undertake the steps mechanically; otherwise, mistakes will occur.

TABLE 1.—*Summary of tests on the effects of hypochlorite solutions on exsheathing, sterility, and development into the 4th stage of* Haemonchus contortus *larvae*

Test No.	Solution	Age of solution in days	Exsheathing in minutes		Dissolution of cast sheaths in minutes		Sterility	Parasitic development in media
			Start	End	Start	End		
1	Labarraque	1	5	8	3	10	+	+
2	"	7	3	5	10	15	+	+
3	"	16	5	10	10	20	+	+
4	"	23	5	8	3	8		
5	"	29	5	10	5	10		
6	"	37	5	15	5	18	+	+
7	"	48	17	20	15	22		
8	"	51	13	20	5	10		
9	"	63	5	10	5	8		
10	"	72	10	14	5	15	+	0
11	"	93	5	8	3	5		
12	"	98	5	8	3	5		
13	"	190	5	8	3	5	+	+
14*	"	59	3	5	5	10	+	+
15	Milton	1	3	5	5	9	+	+
16	"	365	5	8	3	5	+	+
17	Carrel-Dakin	3	10	15	15	35	0	0
18	"	5	10	15	15	30	+	0

* All solutions stored in refrigerator at 5–7° C, except No. 14 held at room temperature, 22–30° C.

Table 1 represents results of work that was done primarily with Labarraque's solution to determine its stability at various ages, at refrigerator temperatures (5–7° C), and at room temperatures (22–30° C). In so far as our work was concerned, the type of stability that interested us was not absolute chemical stability, but power to exsheath, to dissolve the cast sheaths, to sterilize, and to permit the nemas to develop subsequently in media. Later, we were able to procure and compare two lots of Milton's solution with Labarraque, and two tests with Carrel-Dakin's solution were also included.

Many experiments were performed and from these a representative number were chosen for Table 1. It will be seen that no correlation exists between the age of Labarraque's solution and the speed of exsheathing or the dissolution of the cast sheaths. The solutions were

stored in dark bottles and, except in the marked instances, held in the refrigerator. In four tests samples of Labarraque held at room temperatures for from 59 to 64 days were used. One such test is shown in the table and the results are identical with those given above. The data with recent and old Milton are similar to one another and to Labarraque. With Carrel-Dakin's solution the time seems to be considerably extended.

In most of our culture work Labarraque's solution one week old was used and no attempt was made to correlate age with power to exsheath. The table supplies these lacking data. A representative group in this series was carried further, i.e., as regards sterility and ability of the nematodes to initiate development when placed in appropriate media. Sterility of the nemas and their environment was checked initially and again at the end of each experiment. By development in media is meant some degree of growth in the fourth larval (second parasitic) stage (4).

From the data it is seen that Labarraque, stored at 5–7° C, is effective as regards sterility and in permitting development after storage ranging from one day to 190 days. Labarraque held at room temperature for 59 days yielded sterile larvae which developed normally. The same thing obtains for Milton solution held at 5–7° C. In one test with Carrel-Dakin's solution the nematodes were not sterilized and naturally no development occurred. In another test (No. 18) sterility was obtained but no development occurred. However, this test had no control with Labarraque sterilized larvae and the same combination of nutrients, so that the results may not be certain as regards inability to grow after Carrel-Dakin treatment.

SUMMARY

The second ecdysis of certain nematode larvae can be readily produced by placing them in Labarraque's solution at a dilution of 1 to 20 with water. Nematodes so treated include *Haemonchus contortus, Ostertagia circumcincta, Trichostrongylus axei, Obeliscoides cuniculi,* and *Nippostrongylus muris.* The details of the process of exsheathing in Labarraque's solution compare in general with those described by Lapage for Milton's solution. We feel, however, that physico-chemical factors are the most important. A method is described, incorporating the use of Labarraque's or Milton solution, whereby sterile, uninjured nematodes are assured. Labarraque's solution, especially if stored in the refrigerator, remains stable for a long time, at least as regards its power to exsheath and sterilize. Separate lots at different ages have not proved toxic when used at the proper dilution, as evidenced by culture results. In this sense Milton solution is stable also. The results with Carrel-Dakin's solution are uncertain.

BIBLIOGRAPHY

1. LAPAGE, G. 1933 Cultivation of parasitic nematodes. Nature **131**: 583.
2. ——— 1935 The second ecdysis of the infective larvae of certain Trichostrongylidae in solutions of sodium sulphide and of organic compounds containing sulphur. J. Helm. **13**: 103.
3. ——— 1935 The second ecdysis of infective nematode larvae. Parasitology **27**: 186.
4. GLASER, R. W. AND STOLL, N. R. 1938 Development under sterile conditions of the sheep stomach worm *Haemonchus contortus* (Nematoda). Science **87**: 259.
5. GLASER, R. W. AND CORIA, N. A. 1930 Methods for the pure culture of certain protozoa. J. Exper. Med. **51**: 787.

ANAPLASMOSIS TRANSMISSION BY *DERMACENTOR ANDERSONI* STILES

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Anaplasmosis of cattle is a disease which is widespread in tropical and subtropical countries. It was first observed in the United States in southern areas but in 1933 Stiles (1) reported its presence in northwestern Colorado, and in 1935 in Wyoming (2). Various workers have transmitted the disease experimentally with ticks and horseflies; Rees (3) concluded that "ticks are the natural intermediate hosts," whereas Sanborn, Stiles, and Moe (4, 5, 6), who obtained positive results with four species of *Tabanus,* point out that in Oklahoma most cases of anaplasmosis occur during the months when horseflies are the most abundant.

The cases reported by Stiles are interesting in that they occurred within the range of *Dermacentor andersoni.* Male and female *D. andersoni* were collected from a cow that had died of anaplasmosis and forwarded to C. E. Sanborn, Stillwater, Oklahoma, who transmitted the disease to three cows. Since *D. andersoni,* a parasite of large animals in the adult stage, could be an important factor in the spread of the disease within its range, it was believed that additional experiments were justified regarding the ability of this tick to transmit *A. marginale.*

MATERIAL AND METHODS

The experiments were performed at the Oklahoma Agricultural Experiment Station, Stillwater, Oklahoma, in a screened insect-proof barn; furthermore, most of the work was carried on during cold weather when most insects were inactive. No accidental cases of anaplasmosis occurred among the experimental animals. The susceptibility of the test animals was checked by blood inoculation from known carriers.

The purpose of the experiments was, first, to attempt hereditary transmission of the parasites through *D. andersoni,* and second, to obtain additional information on the ability of males to act as vectors. Because of the limited number of cows that were available, there was no repetition of the work of Rees (8), who demonstrated stage to stage survival of the organisms in *D. andersoni.*

The ticks used were part of a laboratory colony.

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* This is a cooperative project conducted by the Oklahoma Agricultural Experiment Station, Stillwater, Oklahoma, and the Bureau of Animal Industry, U. S. Department of Agriculture. L. E. Rozeboom is the Research Entomologist, succeeding C. E. Sanborn; Lewis H. Moe is Station Veterinarian; Geo. W. Stiles is in charge of Branch Pathological Laboratory, Denver, Colorado, Bureau of Animal Industry.

EXPERIMENTAL

A. *Hereditary survival of* A. marginale *in* D. andersoni

1. Survival of *A. marginale* in eggs of *D. andersoni.*

An attempt was made to demonstrate the parasites in the eggs by crushing in sterile physiological saline solution an entire batch of eggs from a female that had fed on a recovered case, and injecting them subcutaneously into a susceptible cow. This animal was kept under observation for almost 4 months but showed no sign of infection. At the end of this period, she was injected with virulent blood and anaplasmosis developed.

2. Experiments with first generation larvae.

Four batches of larvae, approximately 4,000 individuals, were placed on 3 susceptible cows. These larvae were the progeny of 4 females that engorged on a recovered case or on a carrier animal. There was no transmission. The cows were observed for one and one-half to nearly three months before their susceptibility was checked by inoculation of virulent blood. The details of these experiments were not given, as no conclusive results were obtained, due to the failure to recover engorged larvae from the experimental cows. It is possible that some of the several thousand larvae that were given an opportunity to feed actually did engorge, and were overlooked when the bags containing them were removed from the animals' ears.

In one experiment about 300 larvae, from a female that had fed on a recovered case, were ground in sterile physiological saline and injected subcutaneously into a susceptible cow. Results were negative.

3. Experiments with first generation nymphs.

Nymphal *D. andersoni* fed readily on our cows. Approximately 125 nymphs were placed on a susceptible cow, and 59 engorged specimens were recovered. These were progeny of a female that had fed on a recovered case and had been fed on a rabbit in the larval stage. Results were negative. The experimental cow was kept under observation for over two months after the removal of the nymphs before she was injected with virulent blood.

4. Experiments with first generation adults.

Ten females and ten males, progeny of a female that had fed on a recovered case, were placed on a susceptible cow. All of the ticks fed except one female. Only one of the females became fully engorged; the others were partly engorged. Three of the latter were placed in alcohol; the remainder were kept alive and deposited viable eggs. These ticks were fed as larvae and nymphs on a rabbit. Results were negative. The cow was kept under observation for about two months before she was shown to be susceptible by injection of virulent blood.

B. *Transmission by male* D. andersoni

1. On October 14, 1938, a single male tick was removed from the ear sac of cow 211, a recently recovered case. This male tick had been kept on cow 211 for 11 days, and 2 hours after its removal, it was placed in an ear muff on cow 212. The following day this tick was observed to be attached. On October 19, it had detached and was removed. On November 16, suspicious bodies were seen in the red blood cells of cow 212, which were positively identified as *A. marginale* on November 18. On November 23, the cow died of anaplasmosis, constituting a positive transmission by apparently a single feeding of one male *D. andersoni*. This transmission may have been simply mechanical, as only 2 hours elapsed between the removal of the tick from the sick cow and its transfer to the clean animal.

2. On May 31, 1939, a single male *D. andersoni* which had fed on cow 217, a clinical case, was removed from the cow and kept in the laboratory until the following day, when it was placed on bull 222. It fed on the bull until June 6, when it was removed. On July 4, the bull showed a rise of temperature, and blood smears revealed the presence of *A. marginale*. As the tick was kept in the laboratory for a day between feedings, during which time any parasites adhering to the mouthparts might have been destroyed, this perhaps was not simply a mechanical transmission. This experiment shows again that a single male can transmit the disease, but that the transfer need not be an immediate one.

3. On October 3, 1938, male and female *D. andersoni* were placed on recovered case 211; the females were used in the hereditary transmission experiments described above. The males were removed on October 14, and (with the exception of the specimen used in experiment B-1) were kept in the laboratory on moist sand until November 30, when four of them were placed on cow 215. They were observed to be attached the following day, and were removed on December 9, on which date three were still attached, while the fourth had died. On January 7, 1939, *A. marginale* was found in the red blood cells of the cow, and this animal developed a severe case of anaplasmosis, although she recovered. This experiment shows that, once infected, a male tick remains infective for at least 47 days.

DISCUSSION

As *D. andersoni* may be a very important factor in the spread of anaplasmosis within its range, it is desirable to understand more fully the method by which it transmits the disease. One of us (Stiles) diagnosed anaplasmosis in a herd of about 1,000 cattle on June 28, 1939, in the Big Horn Mountains of Wyoming. The first case was noted the last of May and about 100 head had developed the disease. Mortality was estimated at 30 to 35 per cent.

All cattle examined in this region were heavily infested with ticks; from one sick cow, 105 ticks were removed. Approximately 200 adult ticks taken from horses and cattle were all determined to be *D. andersoni.*

Rees showed that nymphal *D. andersoni* can transmit *A. marginale* when infected as larvae, and that adults can transmit the parasites when infected as nymphs. The importance of stage-to-stage transmission by *D. andersoni* is dependent upon the frequency with which immature stages feed on cattle. The normal hosts for these stages are supposed to be rodents. Rees (8) maintains that the apparent absence of ticks on an anaplasmosis case would not necessarily mean that ticks had not caused the infection, since immature stages are very difficult to find on cattle. In another paper Rees (7) points out that Hooker, Bishopp, and Wood fed immature ticks on bovines. Herms and Howell (9) found all stages of *D. occidentalis* on cattle. The bionomics of *D. andersoni* have been thoroughly studied by Hooker, Bishopp, and Wood (10), by Hunter and Bishopp (11), and by Cooley (12). Careful field work led these investigators to conclude that immature *D. andersoni* are normally parasitic on rodents and other small animals, but not on large animals such as cattle. Cooley states that the ticks attack any available animal, but that in general adults attack large animals and immature stages attack small animals. Hunter and Bishopp say that immature stages are mostly found on wild animals, and that *"large domestic animals are the principal hosts of the adult ticks, and the immature stages are rarely, if ever, found upon them."* These authors also point out that although experimentally larvae have been forced to engorge upon cattle, in nature "the larvae feed almost entirely on small wild mammals." If this is true stage-to-stage transmission of anaplasmosis would seldom take place through *D. andersoni,* as nymphs, or both larvae and nymphs, would have to feed on cattle.

Hereditary transmission through *D. andersoni* has not yet been proved, although Sanborn, Stiles and Moe (13) record a suspicious case. Our experiments with larvae were inconclusive, due to the apparent failure of the larvae to feed, but we were unable to demonstrate the organisms in the eggs or larvae by inoculation into susceptible cows. First generation nymphs and adults, progeny of infected females, did not transmit the disease. Rees (7) was also unable to demonstrate hereditary transmission through this tick. The supposed reluctance of larvae to feed on large animals further minimizes the danger of spreading the disease by larvae infected from parent females.

Nymphs and adults of *D. andersoni* readily transmit *A. marginale* by interrupted feedings (13, 14). This species is a three-host, ixodid tick, and ordinarily takes only a single meal in larval, nymphal, and adult female stages, so that in nature transmission by interrupted feeding by any of these stages would perhaps be limited to those instances in which the

original host died, forcing the tick to reattach to another bovine in order to complete engorgement.

Observations by Sanborn, Stiles, and Moe, and the experiments reported here, show that male *D. andersoni* are very efficient vectors of anaplasmosis. Once infected, a male remains infective for at least 47 days and can transmit the disease by a single feeding; it is possible that it could remain infective for a much longer period. If experiment B-1 represents a mechanical transfer, the parasites will remain in a viable state on the mouthparts of a male tick for at least two hours. However, this male had remained on the sick cow for 11 days and on the clean animal for 5 more days, which might be sufficient time for a true biological cycle of the parasites to take place in the tick. In this case, the incubation period in the tick is not more than 16 days.

Sanborn (15), who first reported transmission by *D. andersoni* males, pointed out the possible importance of this stage, which may live for months and will feed many times. Hooker, Bishopp, and Wood (10) state that one of their males "molted between August 30 and September 1, 1909, and died between July 18 and August 18, 1910," a period of between 320 and 353 days. Hunter and Bishopp (11) noticed that males remain on a host for some time and crawl about over the animal searching for females and reattaching at different places. If males were accidentally brushed off an animal, or if the host died, they undoubtedly would seek other hosts. Experiment B–3 shows that males that have fed once can live away from the host, under proper conditions, for at least 47 days. Sanborn transmitted anaplasmosis to at least two cows with his males; there were intervals of one and three days between the removal of the ticks from the infected cows and their application to the clean animals. As Sanborn's ticks remained on the cattle during the entire incubation period, the possibility existed that these males might have reinfected themselves from their hosts, and that their role was merely that of mechanical vectors. Our experiments emphasize the importance of male *D. andersoni* by showing that a single male can act as a vector regardless of whether it passes from an infected animal to a healthy one in two hours, one day, or seven weeks.

SUMMARY

1. We were unable to demonstrate hereditary survival of *Anaplasma marginale* in the eggs or larvae of *Dermacentor andersoni.*

2. First generation nymphs and adults of *D. andersoni,* progeny of females that had fed on a recently recovered case of anaplasmosis, did not transmit the disease by feeding on susceptible cows.

3. Positive results were obtained in three experiments with male *D. andersoni.* In one experiment there was a two-hour interval between

the transfer of a single male from the infected animal to the healthy cow; this male apparently transmitted the parasites by a single feeding. In the second positive experiment a single male that had fed on a clinical case was kept in the laboratory for a day before it was placed on a clean cow. In the third experiment four males transmitted the disease to a healthy animal after having been kept in the laboratory for 47 days between the infective and infecting feedings.

Bibliography

1. Stiles, Geo. W. 1933 Anaplasmosis diagnosed in Colorado. North Am. Vet. **14**: 2.
2. ——— 1935 Anaplasmosis observed in Wyoming. J. Am. Vet. Med. Assn. **87**(N.S. **40**): 579–582.
3. Rees, Charles W. 1934 Transmission of anaplasmosis by various species of ticks. U. S. Dept. Agric. Tech. Bull. 418, 17 pp.
4. Sanborn, C. E., Stiles, Geo. W., and Moe, Lewis H. 1932 Preliminary experiments in the transmission of anaplasmosis by horseflies. Okla. A. & M. Coll. Agric. Exper. Sta. Bull. 204.
5. Rep. Okla. A. & M. Coll. Agric. Exper. Sta. 1932–34, pp. 275–279.
6. Rep. Okla. A. & M. Coll. Agric. Exper. Sta. 1934–36, pp. 42.
7. Rees, Charles W. 1933 The experimental transmission of anaplasmosis by *Dermacentor andersoni*. Parasitology **24**: 569–573.
8. ——— 1930 The experimental transmission of anaplasmosis by *Rhipicephalus sanguineus*. North Am. Vet. **11**: 17–20.
9. Herms, W. B., and Howell, D. E. 1936 The western dog tick, *Dermacentor occidentalis*, Neum., a vector of bovine anaplasmosis in California. J. Parasitol. **22**: 283–288.
10. Hooker, W. A., Bishopp, F. C., and Wood, H. P. 1912 The life history and bionomics of some North American ticks. U. S. Dept. Agric. Bur. Entom. Bull. 106, 165–181.
11. Hunter, W. D., and Bishopp, F. C. 1911 The Rocky Mountain spotted fever tick, with special reference to the problem of its control in the Bitter Root Valley in Montana. U. S. Dept. Agric. Bur. Entom. Bull. 105, 47 pp.
12. Cooley, R. A. 1932 The Rocky Mountain tick. Montana Agric. Exper. Sta. Bull. 268, 58 pp.
13. Sanborn, C. E., Stiles, Geo. W., and Moe, Lewis H. 1938 Anaplasmosis transmission by naturally infected *Dermacentor andersoni* male and female ticks. North Am. Vet. **19**: 1.
14. Boynton, W. H., Herms, W. B., Howell, D. E., and Wood, G. M. 1936 Anaplasmosis transmission by three species of ticks in California. J. Am. Vet. Med. Assn. **88**(N.S. **41**): 500–502.
15. Sanborn, C. E. 1937 Anaplasmosis transmission by *Dermacentor venustus* male ticks. Proc. Okla. Acad. Sc. **17**: 39–41.

SOME OBSERVATIONS ON THE AMABILIID CESTODE, *TATRIA DUODECACANTHA* OLSEN, 1939*

RICHARD L. SCHULTZ

Some 33 specimens of an amabiliid tapeworm, herein identified as *Tatria duodecacantha* Olsen, 1939, were found in the intestine of a pied-billed grebe (the type host), *Podilymbus podiceps* (Linnaeus, 1758), collected near Stillwater, Oklahoma, in August 1939. The worms were fixed in Bouin's fluid, stained in borax carmine, and mounted in toto. When a manuscript in which the parasite was described as new was submitted to the JOURNAL OF PARASITOLOGY, the writer was informed of Olsen's description then in press. Later, a direct comparison of the writer's specimens with paratypes of *T. duodecacantha* revealed the conspecificity of the two lots. In general the author's findings are in complete agreement with those of Olsen. Because of certain differences in interpretation the writer feels justified in offering for publication his drawings (Figs. 1–7) originally intended to illustrate a new species, his comment upon two differences in observation, and a new key to the species of *Tatria* to include *T. fuhrmanni,* unfortunately overlooked by Olsen.

The writer finds both in his own specimens and in Olsen's paratypes that (1) the suckers are armed *only at the margins* and (2) the cirrus of an occasional mature proglottid is protruded into the posterior margin of the adjacent lateral appendage of the preceding segment indicating the occurrence of a posterolateral vaginal pore contralateral to the male pore of the same segment. A vaginal canal, however, leading from this position to the seminal receptacle was never clearly observed.

KEY TO THE SPECIES OF *Tatria*

1. With 10 rostellar hooks 2
   With 12 rostellar hooks ..... *T. duodecacantha* Olsen, 1939
   With 14 rostellar hooks 4
2. Rostellar hooks less than 30 μ long 3
   Rostellar hooks 44–50 μ long ..... *T. biremis* Kowalevski, 1904
3. Guard of rostellar hook longer than handle
   T. decacantha Fuhrmann, 1913

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* Contribution from the Zoological Laboratory, Oklahoma Agricultural and Mechanical College, prepared under the direction of R. Chester Hughes.

The writer is gratefully indebted to Mr. William C. Hill for the collection and preservation of the material, and for the preparation of some of the mounts, used in this study; and to Dr. O. Wilford Olsen of the U. S. Bureau of Animal Industry for the loan of mounted paratypes of *Tatria duodecacantha.*

Guard of rostellar hook shorter than handle
T. appendiculata Fuhrmann, 1908

4. With 7 testes .. *T. acanthorhyncha* (Wedl, 1855) Kowalevski, 1904
With 11–13 "testis lobes" ..... *T. fuhrmanni* Solomon, 1932

The above key is based on information taken from descriptions compiled by Joyeux and Baer (1936) for *T. acanthorhyncha, T. biremis,* and *T. decacantha* and from the original descriptions of the other species.

The name *Tatria scolopendra* (Diesing, 1850) Kowalevski, 1904 is a synonym of *Schistotaenia macrorhyncha* (Rudolphi, 1810) Cohn, 1900 according to Fuhrmann (1932). By mistake *T. acanthorhyncha* was called *T. acanthocephala* by Solomon (1932).

Bibliography

Fuhrmann, O. 1908 Nouveaux tenias d'oiseaux. Rev. Suisse Zool. **16**: 27–73.
————1932 Les ténias des oiseaux. Mem. Univ. Neuchâtel **8**: 1–381.
Joyeux, C., and J. G. Baer. 1936 Cestodes. Faune de France **30**: 1–613.
Olsen, O. W. 1939 *Tatria duodecacantha,* a new species of cestode (Amabiliidae Braun, 1900) from the pied-billed grebe (*Podilymbus podiceps podiceps* (Linn.)). J. Parasitol. **25**: 495–499.
Solomon, S. G. 1932 On a collection of parasitic worms from East Africa. J. Helm. **10**: 209–230.

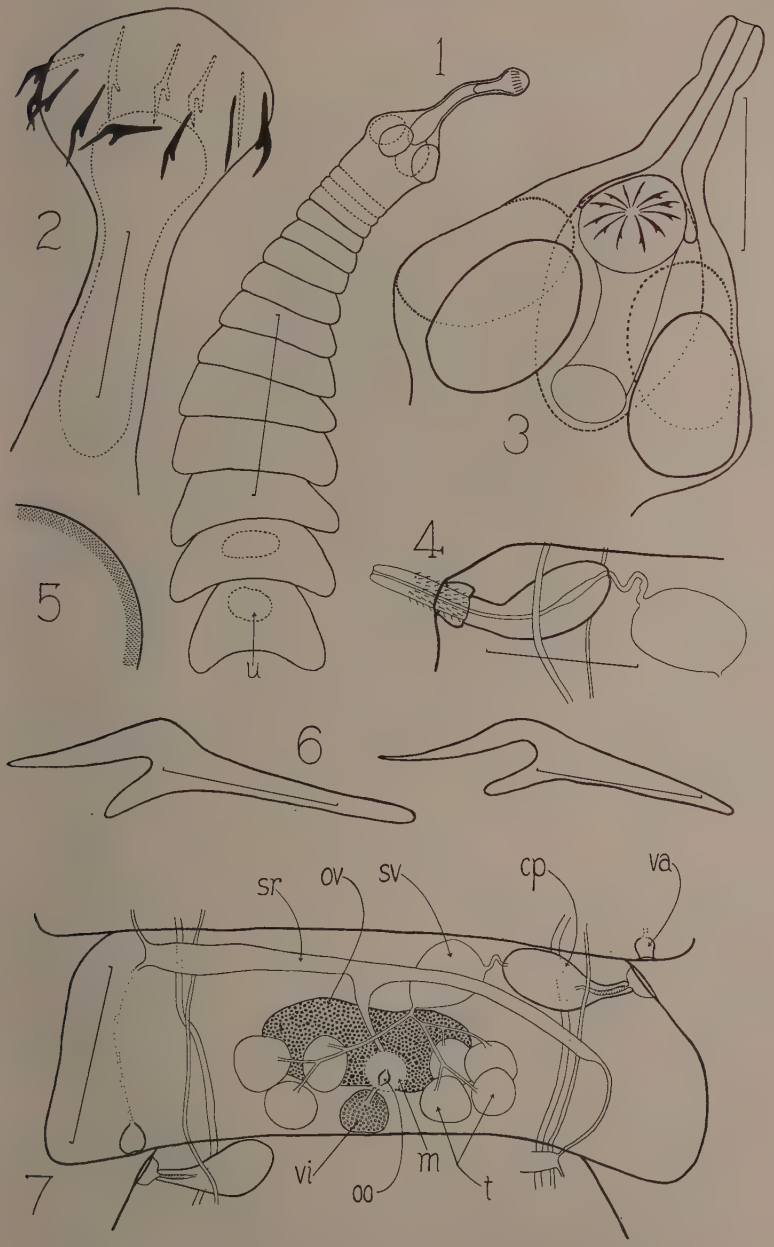
Explanation of Plate

All figures concern *Tatria duodecacantha* Olsen, 1939 and with the exception of 5 and 7 were drawn with the aid of a camera lucida.

Abbreviations

cp—cirrus pouch
m—shell gland
oo—oötype
ov—ovary
sr—seminal receptacle
sv—seminal vesicle
t—testes
u—uterus
va—vagina
vi—vitellarium

Fig. 1. Whole strobila. Scale = 500 μ.
Fig. 2. Everted rostellum. Scale = 50 μ.
Fig. 3. Scolex with retracted rostellum. Scale = 100 μ.
Fig. 4. Cirrus pouch with protruded cirrus. Scale = 100 μ.
Fig. 5. Portion of sucker showing marginal spination.
Fig. 6. Rostellar hooks. Scale = 12.5 μ.
Fig. 7. Mature proglottid, dorsal view. A diagrammatic and composite study. The portion of the vagina shown in dotted lines was never quite satisfactorily observed. Scale = 125 μ.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PYRETHRUM AS AN ASCARICIDE, IN VITRO STUDIES

J. W. Landsberg and N. J. Accousti[1]

In recent years various articles have appeared on the use of pyrethrum, an insecticide, as an anthelmintic. The advantage of using the dried flowers or buds of the chrysanthemum for this purpose lies in their very low toxicity for warm-blooded animals; large quantities may be given orally over extended periods without ill effects. To test the anthelmintic activity, the drug has been given orally and the elimination of the parasites observed (Zunz, 1932; Rebrassier, 1934; Anglade, Gaudin and Arcony, 1931; Pouchet, 1931; Jores and Wolter, 1939), or in vitro studies have been made on pig ascaris (Gaudin and Carrow, 1931; Chevalier, 1930). When given by mouth, pyrethrum has been reported as capable of removing not only ascaris but trichuris, hookworm, taenia, oxyuris and strongyloides from domesticated animals and, in a few instances, eliminating some of these parasites from man.

A search of the existing literature revealed that in no instance had a systematic study of this drug been carried out as recommended by Lamson and Brown (1936) for anthelmintics. Although in vitro studies on the pig ascaris were made by several investigators the methods used were so different that a comparison of the results is virtually impossible. In view of these observations, the authors began a systematic study of pyrethrum. As a starting point, the in vitro method of testing used by Lamson and Brown was utilized in order that the anthelmintic action of this drug could be compared directly with those compounds studied by these authors.

EXPERIMENTAL PROCEDURES

The apparatus used was constructed according to the description of Lamson and Brown (1936) and consisted essentially of a motor driven stirrer and a thermostatically controlled waterbath. The test object, *Ascaris lumbricoides* of swine, was obtained at a local slaughter-house.[2] The worms were tested for motility by plunging them into water at 60° C (24 hours after exposure to the drug) and were considered dead if they displayed no motion in either direction.

The crude drug, consisting of dried powdered whole pyrethrum flowers, was purchased as a one hundred pound lot. Concentrated extracts of the crude drug were prepared by the standard pharmaceutical

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[1] Warner Institute for Therapeutic Research, 113 West Eighteenth Street, New York City.

[2] We are indebted to Mr. S. D. Collins of Adolf Gobel, Inc., New York City, for supplying us with hog ascaris.

procedure of percolation, using different solvents, and concentration. The crude drug (500 grams) was placed in a glass percolator and extracted with the desired solvent (alcohol, chloroform, petroleum ether, tetrachlorethylene) until such time as the percolate was inactive, as determined by the biological assay upon the frog. The fluid percolates thus obtained were vacuum distilled until the solvent employed was removed completely. The remaining residue was a viscous, resinous, dark-green, odoriferous semi-solid (oleoresin of pyrethrum). A part of this oleoresin was used to prepare a purified active principle by the method of LaForge and Haller (1935). The preparation to be studied, suspended in physiological saline, was kept in the incubator at 37.5° C for several hours before testing and its physical state noted.

For each extract of pyrethrum five concentrations of the drug in saline were studied. The concentrations started at 1.0 gm per liter and stopped at 3.0 gm per liter with a determination at each half-gram interval, i.e., 1.0 gm, 1.5 gm, 2.0 gm, etc. For each concentration two runs were made in duplicate (36 worms exposed to each concentration). During each run two ascarids were removed at 2, 5, 10, 20, 30, 45, 60 minutes and at two and three hours. The ascarids were washed in warm saline and stored in the incubator at 37.5° C for twenty hours, after which time they were tested for motility.

EXPERIMENTAL RESULTS

To test the effects of the apparatus upon the parasites, a number of runs were made upon the ascaris in physiological saline alone. The parasites were exposed for a period of four hours (one hour longer than the planned pyrethrum runs) and all were found to be alive; thus the apparatus, per se, appeared to have no injurious effects upon the worms. To check our personal technique, runs were made with substances, in the same concentration, as those used by Lamson and Brown and our results agreed exactly with their published findings for the compounds.

Since the activity of pyrethrum, at least as an insecticide, is dependent upon the pyrethrin content (pyrethrins I and II), an attempt was made to determine the concentration of this constituent in the extracts studied. The original supply of crude drug, as purchased, assayed 0.8 per cent total pyrethrins and upon this basis the total pyrethrin content of the extracts was calculated. The petroleum ether extract contained 26.4 per cent total pyrethrins, the chloroform extract, 32.0 per cent, the alcohol extract, 7.3 per cent, and the tetrachlorethylene extract, 34.8 per cent.

The first runs were made on crude, powdered pyrethrum flowers alone, and no deaths were observed at any of the five concentrations The petroleum ether extract, an insoluble heavy, dark oil, in concentrations ranging from 0.26 gm to 0.79 gm showed no killing power. The

chloroform extract (concentration 0.32 gm to 0.96 gm) likewise was negative. The alcoholic extract (concentration 0.04 gm to 0.12 gm) and the tetrachlorethylene extract (concentration 0.35 gm to 1.04 gm) also did not prove lethal to the ascaris. From each of these prepared extractives a purified product, having a higher pyrethrin content, was made. Runs were made on these purified products at concentrations of 2–1000 which proved negative.

Since none of the extractives, prepared by us, exhibited any killing power in vitro, the question arose as to whether the proper extractive procedures had been followed to obtain the active principle. To answer this question in some measure, a commercially prepared extract, having a pyrethrin content of 27.5 per cent, was purchased and five runs, using the same range of concentrations, were made on it. Negative results were obtained. The results of these runs have been summarized in Table 1. To test further for the presence of the active principle, the frog assay method for pyrethrum, proposed by Rosen and Thompson (1937), was carried out. By this test, in which the frogs become paralyzed, all the extracts and purified extracts were found to contain active pyrethrins.

In view of the fact that the elimination of the parasites had been reported following oral administration of the crude drug, and since this same crude drug had showed no activity in vitro, it was thought perhaps that the alimentary secretions may in some way influence the activity of the drugs. To test this hypothesis, the same concentrations of crude pyrethrum as previously used in saline, were made in artificial gastric and intestinal juice. The former was composed of 0.5 per cent HCl and 0.7 per cent pepsin and the latter contained slightly more than 1.0 per cent bile adjusted to a pH of 8.0 with sodium bicarbonate. The ascarids exposed to the synthetic intestinal juice plus pyrethrum gave negative results; however, some exposed to the gastric juice plus pyrethrum were killed. When the ascarids were exposed to the artificial gastric juice alone, without pyrethrum, it was observed that just as many were killed; thus, the killing action was brought about by the hydrochloric acid and the pepsin rather than the pyrethrum.

DISCUSSION

Unfortunately the present in vitro studies on pyrethrum cannot be compared with those published by other investigators because of the different methods utilized. As pointed out previously, however, our technique has been standardized to conform with that of Lamson and Brown and upon this basis we can compare our results on pyrethrum with their results on other ascaricides. Using a 1–1000 concentration, these investigators observed that hexylresorcinol killed 100 per cent in two minutes,

TABLE 1.—*Summary of in vitro Experiments Showing the Action of Various Extracts of Pyrethrum upon* Ascaris lumbricoides (*pig*)

Extract	Pyrethrin content %	Concentrations studied	Time								
			2′	5′	10′	20′	30′	45′	1 hr.	2 hr.	3 hr.
			Percentage of worms killed								
Crude pyrethrum	0.8	1–1000 to 3–1000	0	0	0	0	0	0	0	0	0
Petroleum ether	26.4	1–1000 to 3–1000	0	0	0	0	0	0	0	0	0
Purified		2–1000	0	0	0	0	0	0	0	0	0
Chloroform	32.0	1–1000 to 3–1000	0	0	0	0	0	0	0	0	0
Purified		2–1000	0	0	0	0	0	0	0	0	0
Alcohol	7.3	1–1000 to 3 1000	0	0	0	0	0	0	0	0	0
Purified		2·1000	0	0	0	0	0	0	0	0	0
Tetrachlorethylene	34.8	1–1000 to 3–1000	0	0	0	0	0	0	0	0	0
Purified		2–1000	0	0	0	0	0	0	0	0	0
Commercial (purchased)	27.5	1–1000 to 3–1000	0	0	0	0	0	0	0	0	0

chenopodium 100 per cent in 30 minutes, thymol 100 per cent in 30 minutes, tetrachlorethylene 100 per cent in two hours and carbon tetrachloride 10 per cent in 3 hours. The pyrethrum preparations, on the other hand, in greater concentrations (ranging as high as 3–1000), showed no killing power in 3 hours. From these comparisons it appears that pyrethrum is not a good ascaricide. One must, however, keep in mind the admonition of Lamson and Brown: "In the testing of any drug one can rely only on the data obtained from the use of this substance against the specific organism under consideration in its natural host."

If we consider the statements in the literature concerning the activity of pyrethrum in vivo in relation to our own in vitro studies we have a situation similar to that described by Lamson and Brown for santonin. None of the ascarids were killed in vitro by this drug in 3 hours (Lamson and Brown, page 100, table 6), but it was effective in removing ascarids from dogs and man. In the opinion of the authors, however, the effectiveness of pyrethrum in the removal of parasites from man and other animals has to be established upon a firmer basis. Most of the reports cite only the elimination of parasites following oral administration of the drug without mentioning any attempt to measure the effectiveness of the treatment, i.e., Stoll egg counts before and after medication. In the work of Rebrassier (1934) on *Ascaridia lineata,* an attempt was made to determine the efficacy of pyrethrum as this author autopsied the test animals following treatment to determine the number of parasites remaining.

Compared with other anthelmintics, on the basis of in vitro studies alone, pyrethrum appears to be a poor ascaricide. Before any final judgment may be made upon the efficacy of pyrethrum, either upon ascaris or any other parasite, it must be subjected to carefully controlled in vivo tests in experimental animals and man.

SUMMARY

Various concentrations of the petroleum ether, chloroform, alcohol and tetrachlorethylene extracts, as well as the crude pyrethrum, were studied, by the in vitro method of Lamson and Brown to determine their ascaricide properties. When compared with other anthelmintics, tested by the same procedure, the pyrethrum preparations were found to have no activity. This observation, however, does not eliminate them completely as ascaricides but indicates further studies adequately controlled, in experimental animals and man.

BIBLIOGRAPHY

ANGLADE, M., GAUDIN, O. AND ARCONY, R. 1931 Sur quelques résults cliniques de l'utilization des pyréthrins dans le parasitisme intestinal et ses troubles secondaires. Bull. Acad. Med. **106**: 654–657.

CHEVALIER, J. 1930 Le pyréthre insecticide; activite pharmacodynamique et therapeutique; culture. Bull. Sc. Pharmacol. **37**: 154.

GAUDIN, O. AND CARRON, B. 1931 Action des pyrethrines sur la musculature des helminthes. Bull. Acad. Med. **106**: 226–229.

JORES, A. AND WOLTER, H. 1939 Über die Verwendbarkeit von Pyrethrum als Anthelminticum. Klin. Woch. **18**: 885–889.

LAFORGE, F. B. AND HALLER, H. L. 1935 Pyrethrins. J. Am. Chem. Soc. **57**: 1893–1894.

LAMSON, P. D. AND BROWN, H. W. 1936 Methods of testing the anthelmintic properties of ascaricides. Am. J. Hyg. **23**: 85–103.

POUCHET 1931 Utilization des pyréthrines en médecine humaine, veterinare et en hygiene. Bull. Acad. Med. **106**: 14–16.

REBRASSIER, R. E. 1934 Pyrethrum as an anthelmintic for *Ascaridia lineata*. J. Am. Vet. Med. Assn. **84**: 645–648.

ROSEN, H. AND THOMPSON, MARVIN R. 1937 The pharmacology of pyrethrum flowers. J. Am. Pharm. Assn. **26**: 631–642.

ZUNZ, E. 1932 Éléments de Pharmacodynamie Spéciale. Paris, France. Masson et Cie.

A NEW GENUS AND TWO NEW SPECIES OF DIGENETIC TREMATODES, WITH A DISCUSSION ON THE SYSTEMATICS OF THESE AND CERTAIN RELATED FORMS

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During our recent routine examination of several specimens of two species of snakes, *Lampropeltis getulus floridana* Blanchard and *Agkistrodon pisciworus* (Lacépède), from Melbourne Prairie, Florida, we have come across many specimens of two species of trematodes that appear to represent a new genus. These are herewith described and their systematic relationship discussed.

Paurophyllum n. g.

Generic diagnosis: Body small, weakly muscular, broader in posterior than in anterior region; cuticula thickly beset with spines throughout; suckers nearly equal in size; ventral sucker in front of body middle; prepharynx present; pharynx muscular, with peri-pharyngeal gland cells; esophagus short, bifurcation well in front of ventral sucker; ceca reaching well behind testes into posterior part of body; genital pore ventral, to one side of midline, midway between ventral sucker and bifurcation; testes smooth to irregular in outline, oblique or with zones entirely separated, close behind ovary; vasa efferentia uniting on entering cirrus sac; cirrus sac moderately developed, reaching to caudal boundary of ventral sucker, curving around side of ventral sucker; seminal vesicle coiled or dilated, filling posterior part of sac; pars prostatica slightly muscular, club-shaped to tubular, short, with numerous prostatic gland cells; cirrus muscular, unarmed; ovary smaller than testes, to one side of midline, at end of cirrus sac; oviduct, oötype, shell gland, and Laurer's canal present; seminal receptaculum present, as large as or larger than ovary, close behind ovary; uterus coiling its way to near posterior margin of body before ascending by same course to genital pore; metraterm muscular, about same length as cirrus sac, with peri-vaginal gland cells; ova fairly numerous, operculated, 40 to 60 μ long, embryonated when oviposited; vitellaria follicular, mainly lateral to ceca, close behind level of ventral sucker; small yolk reservoir present; excretory pore dorsal, near posterior margin of body; excretory bladder I-shaped, with long stem reaching to near level of ovary; flame cell formula of $2\times6\times3$ type. Parasitic in kidneys of snakes.

Type species: Paurophyllum simplexus n. sp.

Additional species: Paurophyllum megametricus n. sp.

Paurophyllum simplexus n. sp.

(Figs. 1 & 2)

Specific diagnosis: Paurophyllum. Body small, weakly muscular, $1.82\pm.04$ (1.58–2.24)* mm long by $0.58\pm.15$ (0.50–0.67) mm wide. Cuticula thickly beset

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* Measurements given in the description of these two species are presented in two ways: 1. The mean with its probable error. In computing the probable error the number of degrees of freedom is taken into account. 2. The range (from smallest to largest) in parenthesis. In the description of each of the two species ten specimens were measured.

with rather heavy spines throughout. Oral sucker subterminal, muscular, 0.18 ± .04 (0.16 – 0.22) mm long by 0.20 ± .04 (0.17 – 0.22) mm wide. Ventral sucker slightly larger, 0.19 ± .04 (0.16 – 0.22) mm long by 0.20 ± .03 (0.17 – 0.22) mm wide, located 0.65 ± .02 (0.44 – 0.76) mm from anterior margin of body. Prepharynx short, pharynx lying close behind oral sucker. Pharynx muscular, 0.095 ± .02 (0.087 – 0.104) mm in diameter, surrounded by rather dense mass of peri-pharyngeal gland cells. Esophagus 0.15 ± .09 (0.087 – 0.200) mm long, rather broad, bifurcating well in front of acetabulum. Ceca slightly dilated tubes, with few irregularities, reaching to within 0.23 to 0.35 mm of posterior margin of body. Genital pore to left of midline, midway between level of acetabulum and bifurcation of ceca, just inside left cecum.

Testes smooth to irregular in outline, often with one or two rather deep indentations in their posterior margins; left testis more advanced, with its anterior margin on level with ovary or up to 60 μ behind that level, more often indented, 0.22 ± .06 (0.17 – 0.28) mm long by 0.18 ± .08 (0.16 – 0.21) mm wide; right testis overlapping zone of left testis, 0.16 ± .10 (0.07 – 0.24) mm behind level of ovary, more regular in outline, 0.23 ± .08 (0.15 – 0.30) mm long by 0.18 ± .05 (0.15 – 0.22) mm wide.

Vasa efferentia arising from anteriodorsal aspect of each testis. Vitellaria follicular, follicles rather large, with 8 to 11 follicles on right side and 9 to 15 on left, mainly lateral and ventral to ceca in area of ovary, from end of cirrus sac to middle of anterior testis. Transverse yolk ducts pass directly in to near midline, forming a small yolk reservoir at uniting.

Ovary smaller than testes, smooth to very slightly irregular in outline, lying to right of midline at end of cirrus sac, 0.14 ± .05 (0.10 – 0.17) mm long by 0.12 ± .04 (0.09 – 0.14) mm wide.

Shell gland conspicuous, in midline close beside inner margin of ovary. Oötype and Laurer's canal present. Seminal receptaculum smaller than ovary, pear-shaped, lying immediately posterior to ovary. Uterus descending between testes to near caudal margin of body, convoluted; ascending by same course to testes where it becomes a straight tube, passing between testes to swing slightly to left of midline and passing to left side or over left half of ventral sucker to genital pore. Metraterm well developed and muscular, in most of the specimens it forms a conspicuous loop anterior to pore before opening to outside, with numerous peri-vaginal gland cells and cuticular inner lining, about as long as cirrus sac, 260 to 330 μ long. Cirrus sac crescent-shaped, curving around right side of ventral sucker, from 260 to 400 μ long, very slightly muscular, containing slightly coiled and much dilated seminal vesicle that fills posterior half of structure, slightly muscular, club-shaped pars prostatica that is surrounded by numerous prostatic gland cells, short ductus, and muscular cirrus. Cirrus unarmed, eversible; in five of the specimens measured the cirrus is everted and in these that organ measures from 130 to 200 μ in length by 60 μ wide.

Ova fairly numerous, operculated, fully embryonated when oviposited, from 42 to 61 μ in length by 23 to 30 μ in width.

Excretory pore dorsal, near caudal boundary of body, guarded by weakly developed sphincter muscle. Excretory vesicle greatly elongated, simple tube, passing anteriorly just under dorsal surface of body to caudal margin of ovary before terminating into two common collecting tubules; the bladder is, therefore, I-shaped. Common collecting tubules diverging laterally, the right passing outward posterior to ovary, passing outward and upward as regular ducts to about posterior margin of ventral sucker, then becoming greatly convoluted ducts to pass up to about level of anterior margin of ventral sucker before swinging sharply outward toward margins of body, near which each gives rise to anterior and posterior main collecting tubules. Each anterior main collecting tubule convolutes its way forward to about level of esophagus where the three accessory tubules are given off in quick succession; the first is short, becomes looped before giving rise to its three capillary tubules, each of which ends in a flame cell in the region of the bifurcation; the second accessory tubule is long and convolutes its way posteriorly to a level

approximating the anterior end of the bladder before forming its group of three capillaries; the third accessory tubule passes anteriorly to form a group of three capillaries in the region of the pharynx and oral sucker. Each posterior main collecting tubule passes posteriorly to about caudal margin of posterior testis before giving rise to accessory tubules, each of which terminate in three capillary tubules that end in a flame cell each; the first group at caudal boundary of posterior testis, the second group midway between that and the posterior end of the body, and the third near the caudal margin of the body. Flame cell formula $2[(3+3+3)+(3+3+3)]$.

Host: Lampropeltis getulus floridana Blanchard.

Habitat: Kidney.

Locality: Melbourne Prairie, Florida, U. S. A.

Type specimen: U. S. Nat. Mus. Helm. Coll. No. 9316. Paratype No. 9317.

Paurophyllum megametricus n. sp.

(Fig. 3)

Specific diagnosis: Paurophyllum: Body small, $1.58 \pm .05$ (1.20 – 1.85) mm long by $0.63 \pm .01$ (0.51 – 0.80) mm wide, weakly muscular, slightly wider in posterior half of body. Cuticula thickly beset with spines throughout. Oral sucker subterminal, muscular, $0.17 \pm .02$ (0.13 – 0.23) mm long by $0.19 \pm .01$ (0.17 – 0.21) mm wide. Ventral sucker muscular, $0.19 \pm .01$ (0.15 – 0.22) mm in diameter, placed from 390 to 530 μ behind anterior margin of body. Prepharynx 34 to 87 μ long, wide, bag-like. Pharynx muscular, $0.08 \pm .01$ (0.07 – 0.10) mm in diameter, surrounded by rather small mass of peri-pharyngeal gland cells. Esophagus from 52 to 95 μ in length, narrow, with slight loop, bifurcating well in advance of ventral sucker. Ceca irregular tubes descending to 0.15 to 0.35 mm of posterior margin of body, with dilations and indentations. Genital pore ventral, to left of midline, midway between bifurcation and anterior margin of ventral sucker.

Testes small, irregular in outline, oblique, with zones abutting or overlapping very slightly, right testis more posterior in position; right testis $0.11 \pm .03$ (0.06 – 0.17) mm long by $0.08 \pm .07$ (0.03 – 0.16) mm wide, placed from almost even with caudal boundary of left to some little distance behind level of left testis; left testis $0.11 \pm .03$ (0.07 – 0.17) mm long by $0.09 \pm .06$ (0.06 – 0.14) mm wide, placed from on level with caudal boundary of ovary to a level 65 μ behind that of ovary.

Vasa efferentia arising from anterior margin of testes, uniting, on entering cirrus sac. Vitellaria follicular, follicles unequal in size, from 7 to 15 follicles on right side and 8 to 15 on left, mainly ventral and lateral to ceca in region of ovary, from end of cirrus sac to anterior margin of anterior testis. Transverse yolk ducts uniting in midline to form small yolk reservoir.

Ovary smaller than testes, $0.08 \pm .03$ (0.05 – 0.09) mm in diameter, often irregular in outline, lying up to 80 μ behind end of cirrus sac.

Shell gland conspicuous, larger than ovary, placed medially, close beside ovary. Oötype and Laurer's canal present. Seminal receptaculum comma-shaped, larger than ovary, lying close behind ovary. Uterus much convoluted in descending between testes to near posterior margin of body; ascending by same course to level of oötype where it joins metraterm; uterus convoluted between and anterior to testes. Metraterm muscular, straight tube except for curving around left side of acetabulum and forming a small, inconspicuous flexure in front of genital pore, $0.26 \pm .04$ (0.18 – 0.33) mm long by 43 μ wide, with numerous peri-vaginal gland cells and rather thick cuticular lining. Cirrus sac rather stout, sausage-shaped structure, slightly curved to pass laterally to right side of ventral sucker, terminating at or just posterior to caudal margin of ventral sucker, $0.29 \pm .03$ (0.21 – 0.36) mm long by 43 to 60 μ wide, containing elongated, coiled seminal vesicle in posterior half, slightly muscular, almost tubular pars prostatica that is surrounded by dense mass of prostatic gland cells, short ductus, and muscular cirrus (in none of our material is the cirrus exserted, but lies coiled within the proximal end of the sac).

Ova fairly numerous, operculated, fully embryonated when oviposited, 43 μ long by 17 to 26 μ wide.

Excretory system identical with that described for *P. simplexus.*

Host: Agkistrodon piscivorus (Lacépède).

Habitat: Kidney.

Locality: Melbourne Prairie, Florida, U. S. A.

Type specimen: U. S. Nat. Mus. Helm. Coll. No. 9318. Paratype No. 9319.

The two species assigned to the genus *Paurophyllum* have many features in common, which at first led to the belief they were identical. However, if we consider their mean measurements it is evident that *P. simplexus* is entirely the larger worm. In *P. simplexus* the large size of the testes and ovary, the proportionately smaller cirrus sac and metraterm, the less muscular metraterm, the uncoiled condition of the uterus anterior to the testes, the regularity of the ceca, the larger and more uniformly distributed vitelline follicles, and the slightly larger ova serve to distinguish it from the smaller *P. megametricus.*

The genus *Paurophyllum* bears a close morphological resemblance to several of the genera of the superfamily Dicrocoelioidea Faust. The genus stands more or less intermediate between *Leptophyllum* Cohn on the one hand and *Styphlodora* Looss on the other. In the opinion of the authors the position of the genital pore in relation to the bifurcation of the ceca and acetabulum, the position of the vitellaria, and the position and arrangement of the gonads in these three genera would indicate a close relationship, and but for the degree of development in the cirrus sac and metraterm, and the finer details of the excretory system they may easily be considered identical.

The genus *Paurophyllum* may be separated from the genus *Leptophyllum* by its more delicate and elongated body, the less pronounced development of the cirrus sac and metraterm, the absence of armature on the cirrus, and the type of excretory system each displays. (We propose to give the details of this system for the genus *Leptophyllum* in the closing section of the present paper.) From the genus *Styphlodora* the present genus may be distinguished by the more pronounced development of the cirrus sac and metraterm, and the type of excretory system described for the two genera.

Recently Byrd et al. (1940) have shown the excretory bladder of the American species of the genus *Styphlodora* to be a greatly evaginated structure with two short cornua and a flame cell pattern of the $2\times6\times3$ type, whereas the present genus possesses a smooth, tubular bladder without cornua and a flame cell pattern of the $2\times6\times3$ type. As will be pointed out presently the genus *Leptophyllum* has a tubular type of excretory vesicle, but in this genus the common collecting tubules arise a short distance before the end of the bladder is reached, and there is a simple arrangement of the main and accessory tubules, quite unlike that

described for the present genus. In the genus *Leptopyhllum* it will be pointed out the accessory tubules arise altogether, a feature not to be found in either *Paurophyllum* or *Styphlodora.*

In certain respects the genus *Paurophyllum* shows morphological relationships to *Astiotrema* Looss, but here the more or less tandem position of the testes, the character of the uterus, and the distribution of the vitellaria prevent further consideration. So far as the writers are aware nothing is known concerning the basic plan of the excretory system in the members of this genus, and until this important system is studied we consider our new genus quite distinct from that genus. In regards to the arrangement of the flame cell groups and the passing of the accessory tubules of the anterior main tubule back toward the posterior part of the body, the excretory system in the genus *Paurophyllum* bears some resemblance to the arrangement of these ducts in the genus *Glypthelmins* Stafford. This resemblance is merely superficial when the details of the excretory system and anatomical features of the body as a whole are taken into account.

Genus *Leptophyllum* Cohn, 1902

The genus *Leptophyllum* was established by Cohn (1902) for the reception of a single species, *L. stenocotyle,* from the rectum of *Herpetodryas fuscus,* a South American snake. In considering the taxonomy of the family Plagiorchiidae, Baer (1924) considered the genus *Leptophyllum* as a *genus incertum* within that family since it showed "remarkably aberrant" characters from any known genus of the group. Later Mehra (1931) followed Baer's example in considering the genus not well enough known to be classified, leaving it in the *genera incerta.* In his more recent article Mehra (1937) apparently has dropped the genus *Leptophyllum* from the family group since no mention of it is made in the discussion or classification of the group. Just recently McIntosh (1939) described the second species, *Leptophyllum tamiamiensis,* of the genus *Leptophyllum.* The species was reported from the uterus and cloaca of the cottonmouth, *Agkistrodon piscivorus,* from the Tamiami Trail, Everglades, Florida. In accordance with the suggestion made by Cohn (1902), McIntosh considered the genus to show a closer morphological relationship to the genus *Enodiotrema* Looss than to the other genera of the dicrocoelid flukes, stating "On account of the heavily armed cirrus (presence of spines not mentioned by Cohn but from his figure they appear to have been present) there appears to be little doubt but that the genus should be assigned to the subfamily Enodiotrematinae Baer, 1924." More recently Byrd and Roudabush (1939) have described a third species belonging to the genus *Leptophyllum, L. ovalis,* from the small intestine of the watersnake, *Natrix taxispilota,* collected at Silver Springs,

Florida. Fortunately, before tendering the description of this species for publication, one of the authors (E. E. B.) encountered over 1500 specimens of *Leptophyllum tamiamiensis* McIntosh. A study of this material has been most timely in determining the details of the excretory system for a member of the genus and in comparing the new species with an already described form. Since the senior author did not have opportunity of examining the living specimens of *L. ovalis,* the details of the excretory system could not be published, nor could we make a definite statement concerning the taxonomic position of the genus.

The material at our disposal, *Leptophyllum tamiamiensis* McIntosh and *L. ovalis* Byrd and Roudabush, conform remarkably to the description of *L. stenocotyle* Cohn, and as suggested by McIntosh they may prove to be identical. The variations from Cohn's species noted for the two more recent forms seem, however, to be of sufficient importance to necessitate the establishment of distinct species. Since *L. ovalis* was described from preserved material, the present authors have had the opportunity of examining living material only in the case of *L. tamiamiensis,* and since the description of that species is adequate, we propose to describe in some detail only the various features of the excretory system in the hope that some light might be thrown on the taxonomic relationship of the genus to its near relatives.

Details of the excretory system of *Leptophyllum tamiamiensis* McIntosh, 1939

The excretory pore opens dorsally near the posterior margin of the body and is guarded by a small, weakly muscular sphincter. From the pore a somewhat flat, tubular excretory vesicle passes anteriorly just under the dorsal surface to the region of the shell gland; its walls are smooth and regular although the vesicle itself may be slightly S-shaped in its course, due to the amount of contraction in the posterior part of the body. Near the anterior end of the vesicle, it is joined by the two common collecting tubules, one from either side. The vesicle is often seen forming a small anterior continuation in front of the point of union of the common collecting tubules, this, however, is not always the case since the common collecting tubules are observed in some to originate from the anterio-lateral margins of the vesicle. The common collecting tubules pass outward and upward, the one on the ovarian side of the body always passes posterior to the ovary while the other may pass anterior to or across the dorsal face of the testis on that side. These tubules, at first, are straight to slightly curving tubes, reaching from the vesicle to about the level of the posterior margin of the acetabulum, then becoming considerably sinuous to coil their way anteriorly to about the level of the anterior margin of the acetabulum. At this point the common collecting

tubules give rise to the anterior and posterior main collecting tubules. Each anterior main collecting tubule coils its way forward to about the level of the genital pore before giving rise to the accessory tubules. Three accessory tubules arise from each main collecting tubule, and these arise simultaneously. In the anterior group on each side, the anterior accessory tubule is long, passing up to the region of the oral sucker before giving rise to three capillary tubules, each of which terminates in a flame cell; the other two are relatively short, one passing forward to about the level of the pharynx before giving rise to its three capillary tubules and flame cells, and the other passing posteriorly to near the level of the acetabulum before giving rise to its three capillaries and flame cells. Each of the posterior main collecting tubules pass posteriorly to about the level of the caudal boundary of the acetabulum, where each gives rise to its three accessory tubules. The posterior one of these is long and passes to near the posterior end of the body before giving rise to its group of three capillary tubes and flame cells; the other two are relatively short, one passing posteriorly to about the posterior margin of the testes and the other passing anteriorly to about the level of the equatorial plane of the acetabulum. Each of these gives rise to a group of three capillaries and flame cells. Flame cell formula: $2[(3+3+3)+(3+3+3)]$. The details of the excretory system are depicted in Fig. 5.

DISCUSSION AND SYSTEMATIC CONSIDERATION

Obviously the general morphology of the members of the genera *Enodiotrema* Looss, *Astiotrema* Looss, *Styphlodora* Looss, *Leptophyllum* Cohn, *Styphlotrema* Odhner, and *Paurophyllum* n. g., as well as others, bear a close morphological relationship. This relationship is less conspicuous when we consider the more detailed accounts of the anatomy of these forms and especially is this true in regards to the excretory system where anything is known concerning that system. As was shown by Looss (1902) the genera *Enodiotrema* and *Styphlotrema* (= *Styphlodora*, in part) show a decidedly different type of excretory system from that described for the genera *Leptophyllum* and *Paurophyllum* in the present paper. In the case of *Enodiotrema*, the excretory vesicle shows definite, lateral outpouchings throughout its course while the cornua form many lateral side branches which in turn may become sub-branched in passing from the end of the bladder, in the region of the gonads, to the level of the oral sucker. The side pouches of the vesicle and the presence of long, well defined cornua, even though these did not show side branches, would exclude that genus from any consideration in the taxonomy of the two genera under discussion. McIntosh (1939) included the genus *Leptophyllum* in the subfamily ENODIOTREMATINAE on the basis of the heavily armed cirrus present in the members of that subfamily and the genus

Leptophyllum. On the basis of the type of excretory system to be found in the two groups we cannot accept this view.

Due to the presence of two rather large, long cornua that encircle the acetabulum in the excretory system of *Styphlotrema* we do not associate this genus with either the genus *Leptophyllum* or *Paurophyllum.* In addition to the anterior cornua, the excretory bladder in *Styphlotrema* gives rise to two rather long, lateral side branches in the region of the pore, giving the bladder the appearance of being a V-shaped bladder on which is superimposed a Y-shaped vesicle. This unusual type of excretory bladder is unique among the trematodes known to us. Recently we have been fortunate in obtaining a number of specimens of *Styphlotrema solitaria* (Looss) through the courtesy of Dr. H. W. Manter, University of Nebraska, one specimen of which clearly showed the characteristic type of excretory vesicle described for this species by Looss. The results of this study have been reported by Byrd, Parker, and Reiber (1940). In the same paper these authors described for the first time the complete details of the excretory system as worked out for certain of the American species of the genus *Styphlodora.* In this genus we find a much dilated vesicle with many and variable outpouchings along the sides and two short, stout cornua. The common collecting tubules arise from dorso-anterio-lateral surface, not from the anterior margin, of the cornua and pass outward for a short distance before giving rise to the main collecting tubules. Although the flame cell formula is of the $2\times6\times3$ type, the accessory tubules are more or less equally distributed along the length of the main ducts. This fact in conjunction with the laterally outpouched vesicle and the presence of cornua would exclude the genus *Styphlodora* from consideration in a taxonomic discussion of the two genera at hand.

The genus *Astiotrema* Looss differs remarkably from *Leptophyllum* and *Paurophyllum* from a morphological standpoint, as pointed out above. Since nothing is known concerning the details of the excretory system for *Astiotrema* and since it bears only a superficial resemblance to *Leptophyllum* and *Paurophyllum,* any consideration of it at this time would contribute nothing to the discussion.

The general morphological details of the two members of the genus *Paurophyllum* very closely parallel those of *Leptophyllum* with few exceptions. Both genera show a general body shape, being more pointed anteriorly and broadening posteriorly to beyond the region of the gonads, then becoming broadly rounded at the posterior extremity. The suckers are typical in that they are about the same distance from each other, the digestive tracts assume the same general pattern, and the genital pore in each genus is located to one side of the midline. The gonads, the uterus, and the vitellaria are comparably situated in respect to the body and to each other. On the other hand they differ from each other in three respects: 1. The absence of cutaneous spines in the genus *Leptophyllum*

and the presence of these in the genus *Paurophyllum*. 2. The presence of an armed cirrus in *Leptophyllum* and an unarmed cirrus in *Paurophyllum*. 3. The degree of development in the metraterm, enormously developed in *Leptophyllum* and well developed, though not to the extent noted for *Leptophyllum*, in *Paurophyllum*. These differences are further augmented by slight differences in the details of the excretory system described for the two genera. Both genera show an excretory flame cell pattern of the $2\times6\times3$ type. The vesicle in *Leptophyllum* sometimes gives rise to the common collecting tubules a short distance back of the anterior margin, whereas these tubules have been observed to originate only from the anterio-lateral margins of the vesicle in *Paurophyllum*. The accessory tubules are described as arising simultaneously from each main tubule in *Leptophyllum*, whereas the proximal accessory tubule of each main tubule arises a short distance from the origin of the other two in *Paurophyllum*. The proximal group of flame cells of the anterior groups is located anterior to the level of the acetabulum and the proximal group of the posterior complement lies adjacent to the acetabulum in *Leptophyllum*, while both of these groups are posterior to the level of the acetabulum in the genus *Paurophyllum*. These differences, both in general morphology and in the excretory system, are held to be of generic value, although the close resemblance between the two genera suggests a close phylogenetic relationship.

As pointed out above both Baer (1924) and Mehra (1931, 1937) have failed to find a satisfactory phylogenetic position for the genus *Leptophyllum*, and the view expressed by McIntosh (1939) concerning the relationship of the genus to the subfamily ENODIOTREMATINAE is not acceptable to us. It, therefore, appears to us that the two genera, *Leptophyllum* and *Paurophyllum*, are without close relationships among the DICROCOELIOIDEA Faust. Due to the close relationship that exists between the two genera, notwithstanding certain points of differences, we propose to create a new subfamily for their reception. Due to genus priority, we propose to make the genus *Leptophyllum* Cohn the type genus of the new subfamily LEPTOPHYLLINAE.

LEPTOPHYLLINAE n. subf.

Subfamily diagnosis: Small, distomate flukes, with or without cuticular spines; suckers equal or subequal, of which the ventral sucker is in or anterior to the body middle; digestive tract consisting of prepharynx, muscular pharynx surrounded by gland cells, short esophagus, and tubular ceca that extend back to or beyond gonads into posterior part of body; genital pore to one side of midline, midway between bifurcation and acetabulum; cirrus sac moderately stout, curving around acetabulum to near caudal boundary of that sucker, containing dilated, coiled vesicula seminalis, slightly muscular pars prostatica, short ductus, and muscular cirrus; cirrus armed (*Leptophyllum*) or unarmed (*Paurophyllum*), eversible; metraterm powerfully developed, muscular, with peri-vaginal gland cells, about as long as or longer than cirrus sac; ovary to one side of midline at end of cirrus sac, smaller than testes; seminal receptaculum large, behind ovary; oötype, shell gland, and Laurer's canal present; uterus descending and ascending between testes, descending to near

posterior margin of body; ova fairly numerous, operculated, fully embryonated when oviposited; vitellaria follicular, mainly lateral to ceca in region of ovary; testes close behind ovary, equal or subequal, opposite or oblique, with smooth to irregular borders; excretory bladder I-shaped, with long stem, giving rise to common collecting tubules close behind level of ovary; common tubules passing upward to level of anterior margin of acetabulum before branching into main collecting tubules; flame cell formula 2[(3+3+3)+(3+3+3)]. Parasitic in digestive, reproductive, and excretory systems of snakes.

Type genus: Leptophyllum Cohn, 1902.

Additional genus: Paurophyllum n. g.

The subfamily LEPTOPHYLLINAE is considered as a subfamily of the family PLAGIORCHIIDAE of the superfamily DICROCOELIOIDEA.

To facilitate a full understanding of the morphological relationship between the members of the genera assigned to the subfamily LEPTOPHYLLINAE we include a sketch of *L. tamiamiensis* McIntosh (Figs. 4 & 5) and *L. ovalis* Byrd and Roudabush (Figs. 6 & 7).

SUMMARY

Two new species, *Paurophyllum simplexus* and *P. megametricus,* are proposed as representing a new genus, *Paurophyllum.* The species *P. simplexus* is described from the kidney of the king snake, *Lampropeltis getulus floridana,* from Melbourne Prairie, Florida, while *P. megametricus* is described from the kidney of the cottonmouth, *Agkistrodon piscivorus,* from the same general area in Florida.

The general relationship the new genus bears to other genera of dicrocoelid flukes is discussed.

The details of the excretory system are described for the members of the new genus, *Paurophyllum,* as well as for a member of the genus *Leptophyllum* Cohn, concerning which very little was previously known.

The relationship of the two genera, *Leptophyllum* and *Paurophyllum,* is discussed in some detail.

A new subfamily, LEPTOPHYLLINAE, is proposed for the reception of the genera *Leptophyllum* and *Paurophyllum.* It is suggested that the subfamily belongs to the family PLAGIORCHIIDAE of the superfamily DICROCOELIOIDEA.

BIBLIOGRAPHY

BAER, J. G. 1924 Description of a new genus of Lepodermatidae (Trematoda) with a systematic essay on the family. Parasitology **16**: 22–31.

BYRD, E. E., M. V. PARKER, AND R. J. REIBER 1940 Taxonomic studies on the genus *Styphlodora* Looss, 1899 (Trematoda: Styphlodorinae), with descriptions of four new species. Tr. Am. Micr. Soc. **59**: (In press).

BYRD, E. E. AND R. L. ROUDABUSH 1939 *Leptophyllum ovalis* n. sp., a trematode from the "brown watersnake." J. Parasitol. **25**: 471–473.

COHN, L. 1902 Zwei neue Distomen. Centr. Bakt. I Abt. Orig **32**: 877–882.

DOLLFUS, R. PH. 1930 Le point d'aboutissement des canaux collecteurs a la vessie chez les Distomes; son importance au point de vue systematique. Ann. Parasitol. **8**: 143–146.

FAUST, E. C. 1929 The trematodes or flukes. Classification. In "Human Helminthology." Lea and Febiger, Philadelphia, pp. 83–93.

——— 1932 The excretory system as a method of classification of digenetic trematodes. Quart. Rev. Biol. **7**: 458–468.

Looss, A. 1902 Ueber neue und bekannte Trematoden aus Seeschildkröten. Nebst Erörterungen zur Systematik und Nomenclatur. Zool. Jahrb. Syst. **16**: 411–912.

McIntosh, A. 1939 Description of a plagiorchioid trematode, *Leptophyllum tamiamiensis,* n. sp., from a poisonous snake. Proc. Helm. Soc. Wash. **6**: 92–94.

McMullen, D. B. 1937 A discussion of the taxonomy of the family Plagiorchiidae Lühe, 1901, and related trematodes. J. Parasitol. **23**: 244–258.

Mehra, H. R. 1931 A new genus (*Spinometra*) of the family Lepodermatidae Odhner (Trematoda) from a tortoise, with a systematic discussion and classification of the family. Parasitology **23**: 157–178.

——— 1937 Certain new and already known distomes of the family Lepodermatidae Odhner (Trematoda), with a discussion on the classification of the family. Z. Parasitenk. **9**: 429–469.

Miller, E. L. 1930 Studies on *Glypthelmins quieta* Stafford. J. Parasitol. **16**: 237–243.

Olsen, O. W. 1937 A systematic study of the trematode subfamily Plagiorchiinae Pratt, 1902. Tr. Am. Micr. Soc. **56**: 311–339.

Explanation of Plate, p. 122

Unless otherwise stated all figures were drawn with the aid of the camera lucida.

AC	acetabulum	OS	oral sucker
AC.T	accessory collecting tubule	P.G	prostatic gland cells
A.M.C.	anterior main collecting tubule	PH	pharynx
		PH.G	pharyngeal gland cells
C	cecum	P.M.T	posterior main collecting tubule
C.P	cirrus pouch		
CP.T	capillary tubule	P.P	pars prostatica
CR	cirrus	P.PH	prepharynx
C.T	common collecting tubule	R.C	right cecum
ES	esophagus	S.G	shell gland
EX.B	excretory bladder	SH.G	shell gland
EX.D	excretory duct	S.R	seminal receptaculum
EX.P	excretory pore	S.V	seminal vesicle
F.C	flame cell	T	testes
G.P	genital pore	T_1	anterior testis
L.C	left cecum	T_2	posterior testis
M	metraterm	UT	uterus
O	ovary	V.G	peri-vaginal gland cells
		VIT	vitellaria

Fig. 1. *Paurophyllum simplexus* n. g. et n. sp. Ventral view of entire specimen, showing the general anatomy.

Fig. 2. Outline drawing of entire specimen of *Paurophyllum simplexus,* showing the details of the excretory system.

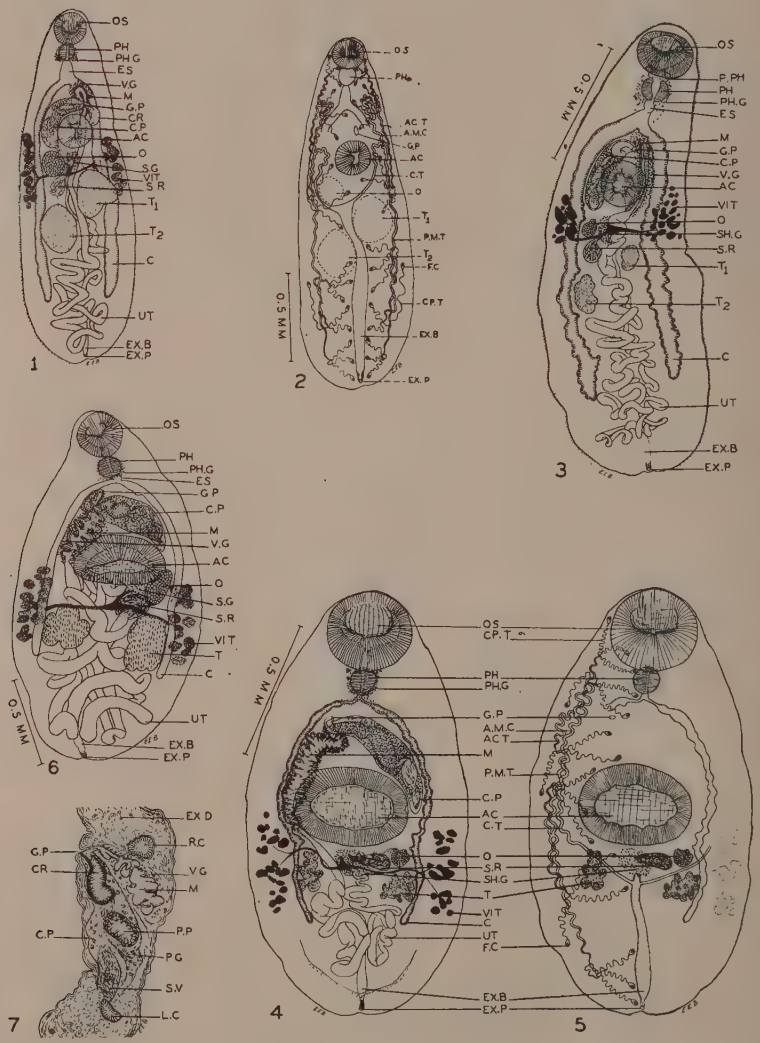
Fig. 3. *Paurophyllum megametricus* n. g. et n. sp. Ventral view of entire specimen, showing the general anatomy.

Fig. 4. *Leptophyllum tamiamiensis* McIntosh, 1939. Ventral view of entire specimen, showing general anatomy. Note the enormous development of the metraterm and the wrinkled condition of the testes.

Fig. 5. Outline drawing of entire specimen of *Leptophyllum tamiamiensis,* showing the details of the excretory system on the right side.

Fig. 6. *Leptophyllum ovalis* Byrd and Roudabush, 1939. Ventral view of entire specimen, showing details of the general anatomy.

Fig. 7. Cross section through the genital pore, cirrus sac, and metraterm of *Leptophyllum ovalis.* ×100.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EXPERIMENTAL INFECTION OF *HYPHANTRIA CUNEA* WITH *NOSEMA BOMBYCIS*[1]

R. R. Kudo and J. D. DeCoursey

Although the majority of parasitic protozoa invade in general a specific host animal, often confining themselves to a particular organ or tissue, there are others which parasitize various organs or tissues of several host species. The microsporidian, *Nosema bombycis,* appears to be one of those which belong to the latter group. This organism has been known to infect and destroy the cells of all tissues of the silkworm, *Bombyx mori,* in all of its developmental stages, and has further been found to parasitize under experimental conditions the larvae of *Arctia caja* (Stempell, 1909), *Margarnia pyloalis* and *Chilo simplex* (Oshima, 1935). The authors have succeeded in bringing about an experimental infection in the larvae of the fall webworm, *Hyphantria cunea.*[2]

The webworm larvae used for the experiment were about 2 cm long, when collected on June 30, 1937, from an elderberry bush in the vicinity of Urbana, Illinois. Ten larvae were dissected and subjected to a thorough microscopical examination, but all were free from any microsporidian parasite. The remaining larvae were removed from the leaves and placed in a battery jar for about 40 hours without food. Spores of the microsporidian[3] were emulsified in distilled water and the emulsion was smeared on young mulberry leaves. On July 2, seven larvae, and on July 9, four more larvae, were allowed to feed on smeared leaves for 30 minutes and then each was removed into an individual vial which was covered with cheesecloth. Each larva was supplied daily with clean leaves. Fifteen larvae were used as controls and were fed exclusively on clean leaves. The eleven spore-fed and fifteen control larvae were given approximately the same amount of food each day and daily microscopical examination of the fecal matter of each larva was made to determine the presence or absence of the microsporidian spores in it.

Fixatives and stains used were the same as those which the senior author has used in his studies on various Microsporidia in the past, including *Nosema bombycis* (Kudo, 1916). A number of section preparations were treated with Feulgen's nucleal reaction.

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[1] Contributions from the Zoological Laboratory of the University of Illinois, No. 538.

[2] The authhors are indebted to the members of Entomology Division of the Illinois State Natural History Survey for the identification of the insect.

[3] Thanks are due Dr. K. Oshima, Imperial Sericultural Experiment Station, Tokio, Japan, for a suspension of heavily infected silk-glands of the silkworm which the senior author received in February, 1936.

The result of the infection experiment is shown in the following table in which + or – indicates the presence or absence of the microsporidian spores in the fecal matter.

TABLE 1.

	Larva No.								
	1	2	3	4	6	7	8	9	11
July 2	Fed on spore-bearing leaves								
3	+	+	+	+	+	+			
4–8	–	–	–	–	–	–			
9	–	–	–	–	–	–	Spores	fed	
10	–	–	–	–	–	–	+	+	+
11	–	–	–	–	–	–	–	–	–
12	–	–	–	–	–	–	–	–	pupa
13	–	–	–	–	–	+	–	–	..
14	–	–	+	+	–	+	–	–	..
15	–	–	killed	+	+	+	–	–	..
16	pupa	+		pupa	killed	+	–	pupa	..
17	..	+		..		+	pupa	..	..
18	..	+		..		+	..	..	..
19	..	+		..		+	..	..	..
20	..	+		..		+	..	..	..
21	..	fixed		..		+	..	..	..
22	..			..		+	..	..	..
23	..			..		+	..	..	..
24	..			..		+	..	..	..
25	..			..		+	..	..	..
26	..			..		o	..	..	..
27	..			..		+	..	..	..
28	..			..		+	..	..	..
29	..			..		+	..	..	..
30	..			..		died	..	..	..
31	adult			..			..	..	adult
August 1				adult			..	adult	
2							adult		

Two larvae are omitted from the table: No. 5 died on the third day (July 4) and No. 10 died on the second day (July 10) of experiment.

Thus it was found that many ingested spores germinated within 24 hours in the mid-gut of the larvae and the empty spores and spores which did not germinate passed out of the alimentary canal completely by the end of that period. From then on for ten days the feces did not contain any spores.

Larva No. 7 began to pass spores in its feces on July 13 and continued to void increasingly more numerous spores until July 30, when it died. Two larvae (Nos. 3 and 4) began to pass spores in their feces on July 14. Larva No. 3 was dissected on July 15. The gut epithelium showed in places advanced infection which explained the appearance of the spores in the fecal matter; various periintestinal tissues were also found to be infected. Larva No. 4 pupated on July 16. Larva No. 6 began to pass spores in its feces on the 13th day after ingestion of the spores and was dissected on July 16. It exhibited conditions similar to those observed in larva No. 3. Larva No. 2 showed spores in its fecal matter on the 14th day and continued to do so until July 20. It was fixed on the following day. Larva No. 10 died on the second day and No. 5 on the third day of experiment. Death in either worm could not be attributed to the microsporidian infection, although the ingested spores were present in the alimentary canal.

Five of the controls died before pupation and all were found to be free from microsporidian infection. There was no recognizable difference in the time of pupation between the controls and the infected larvae, but control adults emerged 3 to 5 days earlier than the infected ones. Four of the infected pupae were enclosed in a thin cocoon and one was entirely naked, being unable to spin a cocoon. The control pupae were fully enveloped in silken cocoons except on the sides that were in contact with the walls of the container.

Larva No. 7 was obviously the most heavily infected. As stated before, this larva began to pass spores on the 11th day after feeding on the spore-smeared leaves. As days passed the larva became sluggish and gradually lost its appetite. It showed the symptom typical of a heavily infected silkworm larva (Kudo, 1924). Unable to spin cocoon, it lived as a larva until July 30, when it died. For several days previous to death the larva did not feed and the feces were watery. The body therefore remained small and showed on its surface numerous spots typical of pébrine disease of silkworm.

Larva No. 2 was studied in serial sections. Schizonts, sporonts, and spores at various stages of development were observed in all tissues. As in the case of silkworm, the silk-glands were extremely heavily infected, both the cytosome and nuclei being highly hypertrophied. Young schizonts were observed in the muscles, gut-epithelium, fat-bodies, and epithelial cells of the silk-glands, Malpighian tubules, tracheae, etc., and much the same as those observed in *Bombyx mori* (Kudo, 1916). Schizogony was binary or multiple fission as in the silkworm. The mature spore contained two independent compact chromatin granules strongly positive to Feulgen's nucleal reaction.

SUMMARY

Infection experiment revealed that *Hyphantria cunea* is easily parasitized by *Nosema bombycis,* when mature spores are taken in per os. Heavily infected larvae show symptoms characteristic of pébrine disease of silkworm. Schizogony and sporogony in *Hyphantria cunea* are similar to those which are known to occur in *Bombyx mori.*

BIBLIOGRAPHY

KUDO, R. R. 1916 Contributions to the study of parasitic Protozoa. I. On the structure and life-history of *Nosema bombycis* Nägeli. Bull. Imper. Sericult. Exper. Sta. Tokio **1**: 31–51.

———— 1924 A biologic and taxonomic study of the Microsporidia. Illinois Biol. Monogr. **9**: 77–344.

OSHIMA, K. 1935 Infection of *Chilo simplex* by *Nosema bombycis* and function of the haemo-lymphocyte. J. Zool. Soc. Japan **47**: 607–626.

STEMPELL, W. 1909 Ueber *Nosema bombycis* Nägeli. Arch. Protistenk. **16**: 281–358.

THE ORIGIN OF HUMAN TAPEWORMS[1]

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The present paper does not propose to deal completely and definitely with the problem of the origin of human tapeworms. It is to be considered as a philosophical essay based on well substantiated facts. The latter are, however, joined together by too many hypothetical links to allow this essay to rank as an hypothesis. The following scientific speculations are the outcome of a study in comparative parasitology, and their chief aim is to show how certain problems can be tackled from an entirely different angle.

Before discussing the various propositions, we must first of all examine from a theoretical as well as from a practical standpoint the argument at the base of this paper, that is, the relationship between the parasite and the host, otherwise known as host-specificity. With regard to the helminths, we have already had the occasion of discussing this problem in a former paper (Baer, 1933) and, while referring to the latter, will make use of the conclusions we arrived at formerly. The question of host-specificity has also been studied among other groups of parasites. Kellogg (1913) has proved its existence among the Mallophaga; Metcalf (1921) and more recently Hegner (1926), discovered it for parasitic Protozoa. It was established several years ago for parasitic fungi and has been evoked more recently by Ch. Nicolle (1934) for the bacteria causing infectious diseases. All these observations and many more tend to prove that host-specificity is a well established fact and not a myth.

Our own research work on Cestoda has convinced us of its existence in that group of parasitic worms. Fuhrmann (1932) has thrown much light on host-specificity among avian tapeworms, which show perhaps the strictest adaptation to their hosts. For mammalian and, to a lesser degree, for fish tapeworms, host-specificity can also be established, although not so distinctly as for birds (group specificity). We have used it as such in collaboration with Ch. Joyeux (1936).

Among the Trematoda, Dubois (1938) has recently shown the importance of host-specificity as an aid to classifying the Strigeida, a group of parasitic worms hitherto considered by most authors as entirely devoid of any specific host-parasite relationships.

The problem of host-specificity, as here examined, can only be applied to adult parasites, larval forms generally showing a much wider range of

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hosts (polyxenous) (vide Baer, 1933). On the other hand, we are perfectly aware that there are exceptions to the rule even after every apparent cause of error has been eliminated. One must also bear in mind that there exist in certain cases so-called "biological parasites," that is, species which are identical morphologically, but which are physiologically distinct (*Hymenolepis nana-fraterna; Ascaris lumbricoides-suis*). To our mind, the species which form exceptions to the rule of host-specificity should be very carefully studied. It would be necessary to prove that a parasite found in a host zoologically removed from its normal host, is able to develop to maturity and produce viable ova or larvae capable of further development. With regard to this statement, we have been able, in collaboration with Ch. Joyeux (1938), to show that the larvae of *Ligula* maintained at 38° C in a medium of physiological salt solution to which was added normal horse serum or ascites liquor, become adult, that is to say, produce eggs. The latter, however, fail to develop further as they are not fertilized, no spermatogenesis having been induced in the tapeworm under these conditions. Results such as these, obtained in vitro in ordinary laboratory experiments, may quite possibly be met with, though their true nature be not recognized, thus leading to a misinterpretation of the facts.

The theory of host-specificity can also be applied to certain problems in parasitology. We were able, some years ago (1925) to estimate the approximate geological age of certain species of CESTODA. It is also possible, knowing the helminthological fauna of a given species of host, or of a group of hosts, to determine approximately their zoological affinities. We have already discussed several such cases elsewhere (Baer, 1933).

The foregoing short summary, for the details of which we refer to the original papers, will none the less suffice to show that the theory of host-specificity can equally well be applied to the tapeworms of man and that interesting conclusions can be drawn therefrom.

What we actually know of the life cycles of the CESTODA and of the anatomy and biology of their larvae, permits us to suppose that, primitively, the ancestors of the CESTODA were parasitic and became adult in aquatic invertebrates. An instance of this possibility is furnished by the genus *Archigetes* which may be considered either as a neotenic larva or as a witness of a long forgotten past.

As time went by, speaking as an evolutionist, the invertebrate hosts of the CESTODA became food for the first predatory vertebrates, that is, the fishes. Consequently, the tapeworm was faced with two alternatives; it was either digested together with its host, or else survived, the latter alone being destroyed. In this case, the tapeworm must be considered as preadapted according to Cuénot (1936) and, coming under the influ-

ence of a new environment, was no doubt subject to anatomical modifications such as the loss of the tail-like appendage bearing the six larval hooklets. Still later, and for reasons we are unable to fathom but which were probably of a physiological order, the tapeworm could no longer grow to maturity within the invertebrate host but only in the fish, and thus the primitive host became the intermediate host.

The origin of a second intermediate host can also be explained in a like manner. The second intermediate host is however not always necessary for the completion of the life cycle; it may on the other hand facilitate the means of spreading the infection (temporary vicarious host) (Joyeux & Baer, 1934). It is thus possible to explain, theoretically, the origin of life cycles with two intermediate hosts such as are actually found among the PSEUDOPHYLLIDEA, which are to be considered as being the forms most closely related to the primitive CESTODA. This the more so, in that in the orders TRYPANORHYNCHA, TETRAPHYLLIDEA and CYCLOPHYLLIDEA, there is to be found a short-circuiting of the life cycle, the second intermediate host being a temporary vicarious host infected by re-encapsulation of the larvae.

We consider that the larval stage known as the procercoid is to be found in all the CESTODA where it subsequently undergoes the change into plerocercoid, plerocercus, cysticercoid or cysticercus larva.[2]

The above hypothesis would explain the long standing association between the definitive and the intermediate hosts. Through physiological segregation during the geological ages there arose gradually an always more intimate relationship between the definitive host and the intermediate host, and consequently also, between the host and the parasite. It is thus possible to imagine the birth of host-specificity, the more strict in that the hosts have become adapted to a particular diet as is the case of birds, for instance. This physiological segregation of the hosts was accompanied by morphological modifications of their anatomy thus forming the basis of our present zoological sub-divisions. The association of physiological segregation on the one hand and of morphological differentiation on the other, go to form what we are accustomed to term *evolution*.

All that is actually known of the CESTODA leads us to consider them as having arisen from a common stock (monophyletic), their subsequent

[2] The procercoid larva as discovered and described for the first time by Rosen and Janicki (1918) appeared as an entirely new larval form. Further research work however on other species of the PSEUDOPHYLLIDEA and also on the TETRAPHYLLIDEA, has shown that this larval form is hardly ever so well differentiated as in *D. latum*, especially with regard to the posterior hook-bearing appendage (cercomer). We are perfectly justified in considering as the procercoid stage, the larval period situated between the moment the hexacanth embryo penetrates into the tissues of the intermediate host, and the ensuing larval transformation into a plerocercoid, plerocercus, cysticercoid or cysticercus.

morphological differentiation having appeared simultaneously with that of the vertebrates, their hosts. Consequently, the CESTODA also contribute their share in favor of the evolutionary doctrine.

In view of the above hypotheses, one must consider helminths which show a lower degree of host-specificity (NEMATODA, for instance), as geologically more recent than the CESTODA or certain groups of TREMATODA, the monophyletical origin of the latter having not yet been proved. The same theoretical views give us a clue as to why the helminths of domestic animals generally show a marked lack of host-specificity owing to the fact that through the intervention of man, their internal metabolism has been altered and consequently differs considerably from that of the corresponding wild species.

Let us now apply the above data and theoretical views to the tapeworms of man. Nearly thirty species of human tapeworms have been described from all over the world, yet only two or perhaps three species are found exclusively in man and have not been obtained fully mature in other experimental hosts. These species are *Taenia saginata* (Goeze, 1782), *Taenia solium* Linnaeus, 1767, and perhaps also *Raillietina* (*Raillietina*) *demerariensis* (Daniels, 1895). All the other known species are either shared in common with wild or domestic animals or else are synonyms. Most of these latter species are rare human parasites and should be considered as erratic in man (vide Joyeux & Baer, 1929). An exception should however be made for the species *Bertiella studeri* (Blanchard, 1891) found simultaneously in man and in monkeys. This species is perhaps the only one to have been acquired by the prehominian ancestors of man before the Ice-Age. It has subsequently become localized in subtropical climates although such climates do not appear to be necessary for the completion of the life cycle. Stunkard (1939) has shown that ordinary free-living mites such as are found in northern Europe, can be infected experimentally with larvae when fed mature ova. Our prehominian and even our hominian ancestors could consequently have acquired their tapeworm infection long before they became either hunters or fishermen. The actual status of the problem of host-specificity urges us to state that in all probability *B. studeri* is the only original tapeworm acquired by man through evolution. The scarcity of this species today as a human parasite, outside of laboratories working with monkeys, can be easily explained by the "domestication" man has undergone since, with the result that his habits now differ widely from those of monkeys and consequently his chances of contamination by the latter are reduced accordingly.

Diphyllobothrium latum (Linnaeus, 1758) is another species of human tapeworm which should be examined separately. This species is endemic wherever its life cycle can be completed, i.e., almost exclusively around

the lakes of the northern hemisphere. This human tapeworm is frequently found as a normal parasite, in several domestic and wild carnivora and also in the pig.[3] Vogel (1929) succeeded once in infecting a monkey, but the latter shed its worm spontaneously (abnormal host). The experimental infection of monkeys is altogether exceptional as already observed by LeBas (1924) and Eguchi (1926) (fide Vogel, 1929); we ourselves have failed on two occasions. On the other hand, and in spite of classical text-books, the dog and the cat can always be infected without the slightest difficulty whatever. We have even obtained by male-fern treatment from a dog, a specimen of *D. latum* 20 mm broad, which is thus wider than the human specimens, 10–12 mm. Such cases are, however, exceptional.

The presence of *D. latum* in man and in domestic as well as in wild carnivora in the United States and Canada, leads us to consider the part played in the introduction of this worm by the European immigrants as being of secondary importance (Vergeer, 1929). We are perfectly justified in admitting that the original source of infection in North America came from Asia, via Siberia, either with the migration of the carnivora or perhaps also with the migration of the primitive Siberian tribes. Unfortunately, we know next to nothing of the parasitic fauna of the North American Indian, a probable descendant from these immigrants.

We personally consider *D. latum* to be a tapeworm of carnivora with fish-eating habits, which has become adapted secondarily to man. The latter first infected himself when he started to fish, eating his catch raw, that is to say, fairly recently in the history of humanity.

The presence of *D. latum* in the pig has so far only been observed in Carelia (Russia). It is of interest to note that there appears to exist without any doubt, a physiological similitude between man and the pig, one of the consequences of which is that they share identical or very closely related parasites (*Balantidium, Ascaris*). This has no doubt originated through both of these hosts being omnivorous, with the pig, domesticated by man, fed largely on scraps of human food.

Several tapeworms of rodents are also capable of developing in man, this being the case for certain species of the genus *Raillietina,* for *Hymenolepis diminuta* and for the much discussed species *H. nana-fraterna*. This latter species is of special interest owing to its close adaptation to the human organism insomuch that *H. nana* from man has become physiologically segregated from *H. fraterna* from rodents. This segregation may possibly have been brought about in a relatively short time owing to the fact that this species of tapeworm does not normally require an intermediate host. On the other hand, there also appears to be a certain

[3] Meggitt (1924) gives a list of PINNIPEDIA as hosts for *D. latum*. However, *D. latum* so far has never been recorded from seals. This mistake is due to a mistranslation of Max Braun's German text.

physiological relationship between man and mouse which is made evident by common tapeworm parasites and also by the fact that viral diseases, such as yellow fever and others, can be transmitted from man to mouse. This might indicate the presence of a certain degree of preadaptation of the murine parasites to the human organism. The presence of *H. fraterna* almost exclusively in "domestic" rodents, i.e., associated with man, would indicate that the original infection was contracted at a period when man first began to lay in stores of food and corn, that is, when he abandoned his nomadic habits to settle down and indulge in primitive husbandry.

Raillietina (*Raillietina*) *demerariensis* (Daniels, 1895) has been made the subject of a recent paper (Joyeux & Baer, 1940) and is not yet well enough known, faunistically speaking, to be of much use in our present essay. This species, closely related to forms from the Far East, might perfectly well have been brought to Central and South America by the ancestors of the actual population who came over from the Far East, as modern ethnological studies tend to prove. The segregation of this tapeworm relatively long ago, and the absence of rats (since imported) in these countries, the former being the normal hosts in the Orient, have isolated this species in man and have brought about slight modifications of the internal anatomy and in the size of the worm, which distinguish it from the oriental forms. *R. demerariensis* is probably a recent human parasite.

There now remain to be examined the two largest species of human tapeworms, *Taenia saginata* (Goeze, 1782) and *Taenia solium* Linnaeus, 1767. All the known species of the genus *Taenia* s. str. are parasitic in carnivora, the larval forms being found in herbivorous (or omnivorous) animals. These species apparently show a marked host-specificity, the species from the dog being distinct from those from the cat or from the weasel. The only two known species from man have not yet been obtained fully mature from experimental animals which would seem to indicate clearly that these species are true human parasites.[4]

If we apply the different hypotheses proposed above to these two human tapeworms, we can state positively that if man had acquired these worms via what we will call the *"evolutionary route,"* closely related species should be found today in the higher primates. This we know, however, is not the case, and what is more, so far nobody has succeeded in infecting primates experimentally.

We consider then that man has acquired these worms secondarily and

[4] A partly adult *T. solium* was once recovered by Schwartz (1927) from a dog infected with *C. cellulosae* and Faust (1930) reported ripe segments obtained from a monkey fed on the larvae of *T. confusa* (= *T. saginata*). Neither of these cases has however been verified experimentally since, and from what we know, we consider these results as exceptional.

by accident. It is almost certain that the Summoprimates (Weinert) from which descend our hominian ancestors, were vegetarians and had not yet reached the higher stage of hunters and, consequently, flesh-eaters. We do not wish to overstep the compass of the present paper and discuss in what manner the taste for raw flesh was acquired by man. Psycho-paleontology is as yet unborn! We are, however, perfectly justified in supposing that the larger extinct carnivora, such as *Machairodus,* the cave-lion, etc., which were contemporary to the hominian, could readily have harbored adult tapeworms the larvae of which lived in wild pigs, the latter being hunted by our ancestors. In this way, the infection of man with *T. solium* was realized and could perfectly well have been maintained in the human species, since the latter serves also as intermediate host, and the chances of fecal contamination must have been considerable. It is certain that cannibalism was prevalent among our distant ancestors, either from lack of fresh meat or for religious purposes. Man of the mesolithic period, following the Ice-Age, domesticated the dog thus introducing a new intermediate host, since the dog was also destined to be eaten. The pig, domesticated at a much later period, lived freely among the human agglomerations and had every chance of becoming "measly." In this way, man succeeded in accumulating, unwittingly, within the area of his tribe, both the animals serving as intermediate hosts and the maximum chances of realizing the life cycle of the tapeworm.

We can apply the same process of reasoning to the case of *Taenia saginata* with the restriction that the larval form of this species is hardly ever found outside the herbivora. In this case, man first realized the most favorable conditions for reinfecting himself only after he had domesticated these animals, especially the bovines. This stage was reached by our ancestors at a period extremely remote when viewed from the present day, yet this period is more recent than that evoked for the origin of *Taenia solium* in man.

The possibility of adult tapeworms of carnivora becoming established in man is still shown today by the species *Dipylidium caninum* (Linnaeus, 1758) which appears to be frequently transmitted by man's domestic pets, the dog and the cat.

This short biological sketch, leads us to the following conclusions. *B. studeri* is the only tapeworm acquired by man's distant ancestors, having followed the latter throughout their evolution. *D. latum* was acquired by primitive man when first he began to fish, eating raw his catch contaminated with the larvae derived from the life cycle of adult worms living in wild carnivora. The complete absence in primates of species belonging to the genus *Taenia,* led us to conclude that primitive man acquired his worms accidentally from the carnivora then living.

Cannibalism on the one hand and domestication of the dog and much later of the pig, on the other, explain how our ancestors maintained their infection with *T. solium*. *T. saginata* must have been acquired at a later period when man became first a hunter, then a cattle-breeder.

Bibliography

Baer, Jean G. 1925 Some Cestoda described by Beddard, 1911–1920. Ann. Trop. Med. and Parasitol. **19**: 1–22.

———— 1933 L'adaptation des Helminthes à leurs hôtes. Bull. Soc. Neuchâtel. Sc. Nat. **58**: 57–76.

Cuénot, L. 1936 L'espèce. 310 pp. G. Doin & Cie. Paris.

Dubois, G. 1938 Monographie des Strigeida (Trematoda). Mém. Soc. Neuchâtel. Sc. Nat. **6**: 535 pp.

Faust, E. C. 1930 A study of the rare human tapeworm, Taenia confusa, with a report of the fourth case. Southern Med. J. **23**: 902–906.

Fuhrmann, O. 1932 Les Ténias des Oiseaux. Mém. Univ. Neuchâtel **8**: 381 pp.

Hegner, R. 1926 The biology of host-parasite relationships among Protozoa living in Man. Quart. Rev. Biol. **1**: 393–418.

Joyeux, Ch. & Baer, J. G. 1929 Les Cestodes rares de l'Homme. Bull. Soc. Path. Exot. **22**: 114–136.

———— 1934 Les hôtes d'attente dans le cycle évolutif des Helminthes. Biol. Méd., Paris **24**: 1–25.

———— 1936 Cestoda. Faune de France **30**: 610 pp.

———— 1938 L'évolution des plérocercoïdes de la Ligule intestinale. C. R. Soc. Biol. **129**: 314–315.

———— 1940 Anatomía y posición sistemática de Raillietina (Raillietina) quitensis León, 1935, Cestode Parásito del hombre. Rev. Med. Trop. y Parasitol. Bact. Clin. y Lab. (in press).

Kellogg, V. L. 1913 Distribution and species forming of ecto-parasites. Am. Naturalist 129–158.

LeBas, G. Z. L. 1924 Experimental studies on Dibothriocephalus latus in Man. J. Helm. **2**: 151–166.

Meggitt, F. J. 1924 The Cestodes of Mammals. London.

Metcalf, M. 1921 The geographical distribution of the Anura and their Opalinid parasites. Anat. Rec. **20**.

Nicolle, Ch. 1934 Destin des maladies infectieuses. Paris, Alcan éd.

Rosen, F. & Janicki, C. 1918 Le cycle évolutif du Dibothriocephalus latus L. Bull. Soc. Neuchâtel. Sc. Nat. **42**: 19–53.

Schwartz, B. 1927 Note on Cysticercus cellulosae. Proc. Helm. Soc. Wash., J. Parasitol. **14**: 203.

Stunkard, H. W. 1939 Observations on the development of the Cestode *Bertiella studeri*. 3rd. Int. Cong. Microbiol., New York, 179. (Abstract)

Vergeer, T. 1929 The Broad Tapeworm in America. J. Inf. Dis. **44**: 1–11.

Vogel, H. 1929 Helminthologische Beobachtungen in Ostpreussen, insbesondere über Dibothriocephalus latus und Opisthorchis felineus. Deutsch. Med. Woch. **39**: 1–7.

THE LIFE CYCLE OF *POSTHARMOSTOMUM GALLINUM*, THE CECAL FLUKE OF POULTRY[1]

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Postharmostomum gallinum (Witenberg, 1923) is a parasite known to occur in chickens in Hawaii. It is especially common in birds raised on the ground in areas where the land snails, *Eulota similaris* and *Subulina octona,* occur. The fluke was originally reported from this locality by Guberlet (1928) under the name of *Harmostomum* (*Postharmostomum*) *hawaiiensis,* but it is now generally regarded as *Postharmostomum gallinum.*

Early in 1938, while dissecting several land snails (*Eulota similaris*) collected on a poultry farm, the writer encountered in the pericardial cavities adolescariae possessing characters of those found in the BRACHYLAEMIDAE. When these adolescariae were fed to laboratory-raised chicks, they developed to the adult stage in the ceca and were identified as *Postharmostomum gallinum.* This observation led to an investigation to determine the complete life cycle of the fluke, and preliminary reports relative to this study were made by the writer in 1938. In the same year Fukui and Ogata reported that Ozaki found agamodistomes in the pericardia of *Euhadra peliomphala* and *Phylomycus bilineatus* and reared them to adult *"Harmostomum gallinum"* in fowls.

The present paper reports, among other things, more details on the development of *Postharmostomum gallinum* in the intermediate and final hosts.

MATERIAL AND METHODS

In order to insure freedom from natural fluke infections, land snails, *Eulota similaris,* were raised under laboratory conditions. Eggs of these snails, collected from damp areas under rocks, were placed in large glass jars containing soil, dead tree leaves, and small rocks; this material had been collected away from poultry farms. The jars were kept covered with a double layer of cheesecloth, and the contents were sprinkled with water occasionally to keep them slightly moist. When the eggs had hatched, pieces of lettuce were added to the contents of the jar, to serve as additional food for the growing snails.

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Snails of about 4 to 5 mm were experimentally infected by allowing them to feed on freshly dissected out uteri of flukes containing eggs. A few snails were placed with a uterus in a little water in a Petri dish. All the snails which had been observed to feed on fluke eggs were segregated in jars with proper soil and food and killed at various intervals. Other land snails, *Subulina octona,* were fed fluke eggs in the same manner. *Eulota similaris* and *Subulina octona* were the only snails used in these experiments for the reason that they are the two land snails commonly found in local poultry yards, and the findings would therefore have a definite bearing on the control of the parasite in this locality.

Snails were infected with cercariae, for study of development of the adolescariae, by breaking up the walls of fully grown sporocysts, liberating their contents, and allowing the snails to come in contact with the cercariae thus obtained as described in more detail later.

For study of development in the final host, adolescariae were fed to 6- to 8-week-old laboratory-raised chicks. Experimentally infected birds were kept in the laboratory in suitable cages and killed about a month after infection. One uninfected bird was placed in each cage to serve as a control.

GENERAL DESCRIPTION OF DEVELOPMENTAL STAGES

Egg and miracidium (Fig. 1): Shell of egg dark brown, ovoid and slightly asymmetrical, 37 to 39×18.5 microns. Miracidium within egg shell fully developed when oviposited, about 25×15 microns; most part of body covered with cilia, with exception possibly of anterior fourth of body which could not be clearly studied. Anterior end directed towards operculum of eggshell. Ocelli absent; internal morphology not very distinct, but organism appears to be made up of few cells.

Sporocyst (Figs. 2, 3, 4, and 5): Sporocyst branching in type, consisting mainly of central body from which radiate about 4 to 6 main branches; each branch may give rise to short secondary branches. Main branches may measure up to 4 mm ×0.1 mm. Terminal tip of branches provided with external opening, with a muscular wall which appears to serve as a birth pore. Germ balls of various stages of development up to and including cercariae, usually numerous in body cavity. Redia stage absent.

Cercaria (Figs. 10, 11): Fully developed cercaria in sporocyst or in water outside of snail host, active or temporarily inactive. When found inactive in water, the body becomes slightly contracted and the anterior portion is usually bent slightly ventrad (Fig. 10). In moderately extended position, body about 186 to 193×75 to 80 microns. Many minute spines present in posterior tip of body, and few scattered ones in various portions of body. Oral and ventral suckers each about 82 microns in diameter, the ventral sucker about 30 to 35 microns posterior to oral sucker. Pharynx about 22×15 microns. Esophagus apparently absent. Ceca saccular, lateral, usually extending slightly posterior to ventral sucker. Genital primordia visible, slightly below ventral sucker. Excretory bladder sends out two short lateral excretory tubules, each opening laterally at posterior portion (Fig. 12).

Adolescaria (Fig. 15): Adolescariae recovered from pericardial cavity of snail host and fixed in Gilson fluid, measure about 790 to 970×270 to 390 microns. Oral and ventral suckers, 125 to 175 and 80 to 140 microns in diameter, respectively. Pharynx 67 to 100×67 to 81 microns. Ceca sinuous, extending to posterior portion of body. Anlage of testis visible about center of posterior fourth of body, but other genital structures not well differentiated. Excretory bladder opening by a central pore slightly dorsal to posterior end; excretory pore apparently representing

opening of base of excretory bladder formed as result of pinching off and degeneration of posterior tip of body which in the cercaria stage contained the two excretory tubules (Figs. 13, 14).

Development in the intermediate host

Development of the sporocyst and cercaria. On April 15, 1938, several snails, *Eulota similaris,* were fed eggs of *Postharmostomum gallinum* obtained from the uterus of live adult flukes. Feces of these snails examined about 15 hours later contained several empty fluke-egg shells, indicating that the miracidia had escaped in the intestinal tract of the snails within that period. The snails were kept in suitable jars and dissected at regular intervals following infection, with results as follows:

(a) May 5, 20 days following infection, each of two snails dissected contained in the liver several young slightly motile sporocysts showing several buds or branches originating from the central portion of the body (Figs. 2, 3). The total structures varied from about 200 to 520 microns in maximum length. Several germ balls were enclosed within each sporocyst.

(b) May 25, 40 days following infection, each of two snails dissected contained in the liver several branched sporocysts (Fig. 4); the branches varied from about 120 to 800 microns in length. Each sporocyst enclosed several germ balls of various degrees of development up to young cercariae with well differentiated oral and ventral suckers. No rediae were noted.

(c) June 14, 60 days following infection, each of two snails dissected contained several branched sporocysts (Fig. 5); the main branches varied from about 300 to 1200 microns long. The sporocysts contained, for the most part, developing cercariae (Figs. 6, 7, 8, 9), a few of which appeared to be fully mature. In one of these snails two cercariae were found also in the kidney cavity, indicating possibly a recent migration. (See discussion.)

(d) July 14, 90 days following infection, one snail contained several branched sporocysts; the main branches were in most cases tangled, but most of them measured from 1 to 2 millimeters in length. A large number of mature cercariae were enclosed in each sporocyst. A few developing adolescariae were also present in the pericardial cavity of the snail.

Evidence that cercariae leave the body of the snail host was obtained by placing the above-mentioned snail before dissection in a Petri dish half filled with water; during the course of 3 hours four cercariae were noted crawling on the bottom of the dish.

Development of the adolescaria

The fact that further development of the mature cercariae takes place in the pericardial cavity of the snail, and postulating that this cavity is

most probably reached through the external renal aperture, it was believed that snails could be experimentally infected if allowed to come in contact with free cercariae. This was successfully accomplished by placing several snails, *Eulota similaris,* in a Stender dish containing a large number of cercariae in a few drops of water. Following a few hours exposure to infection, the snails were isolated in suitable jars and dissected at various intervals as indicated below.

On August 2, about 15 hours following exposure to infection, two snails were dissected and showed one and two cercariae respectively in their pericardial cavities; no change was noted in the size and development of the cercariae. Another snail dissected on the twelfth day after exposure harbored in the pericardial cavity one developing adolescaria which measured 491 × 200 microns (Fig. 13). A conspicuous feature was the apparent pinching off and partial degeneration of the posterior tip of the body formerly containing the two lateral excretory ducts.

Of two additional snails dissected 25 days following exposure to cercarial infection, one was negative and the other contained in the pericardial cavity four developing adolescariae, all of uniform size. One of these measured 825 × 253 microns. At the posterior tip of the body of each of these four organisms there remained attached, by a fine connection, a small sac-like structure about 35 microns long (Fig. 14), which apparently represented the remains of the degenerated posterior tip of the body. These organisms at this stage may, however, be considered as fully developed adolescariae and possibly infective, since they had acquired an appearance characteristic of that stage.

Development in the final host

On April 13, 1938, a 6-week-old cockerel was fed 50 land snails, *Eulota similaris,* collected in a poultry farm in which *Postharmostomum gallinum* was known to occur. The examination of the pericardial cavities of a few of these snails revealed them to be naturally infected with brachylaemid adolescariae, the specific identity of which was at that time not known to the writer. About a month later (May 15) the bird was killed and at autopsy eight adult flukes, identified as *Postharmostomum gallinum,* were found in the ceca.

On the same day that the above-mentioned bird was fed adolescariae, another 6-week-old cockerel was fed several branched sporocysts containing apparently mature cercariae; the sporocysts in this instance had been isolated from a naturally infected snail, *Eulota similaris.* When the bird was killed a month later no flukes were found in the ceca, suggesting that the cercarial stage was not infective to birds. Two control chicks kept with the experimental birds also were negative for flukes.

On September 25, 1938, a 6-week-old chick was fed five snails,

Eulota similaris, the latter having been experimentally exposed to cercariae of *Postharmostomum gallinum* about 55 days previously. Just before feeding one of the snails was dissected and found to contain adolescariae. On October 26, the bird was killed and was found to harbor in the cecum one adult fluke measuring 8×2 mm. A bird used as a control in this experiment remained free of all helminths.

On December 30, 1938, each of two 2-month-old pullets was fed 50 snails, *Eulota similaris,* naturally infected with adolescariae of *Postharmostomum gallinum.* Following 15 days of infection, the cecal droppings of these birds were examined daily for fluke eggs; these examinations revealed the presence of fluke eggs on January 23 and 26, respectively. On January 30, 1939, the birds were killed and at autopsy several adult *Postharmostomum gallinum* were found in the ceca. Considerable inflammation and blood were also noted in the ceca. Evidence of blood in the cecal droppings of these birds about 2 weeks after infection is of interest since these birds did not suffer from coccidiosis or other cecal parasitism. As two control birds did not show bloody droppings, it is believed that the flukes were responsible for the pathology of the ceca.

On September 28, 1939, each of two 2-month-old cockerels was fed 50 snails, *Subulina octona,* naturally infected with adolescariae believed to be those of *Postharmostomum gallinum.* Fluke eggs were found in the cecal droppings of these birds on October 26. On October 29, the birds were killed and at autopsy revealed one and two adult *Postharmostomum gallinum,* respectively, in the ceca.

DISCUSSION

The observations reported in this paper indicate that the development of *Postharmostomum gallinum* in the snail intermediate host involves the development of (a) the sporocyst and cercaria in the liver, and (b) of the adolescaria in the pericardial cavity. The cercaria, to develop to the adolescarial stage, leaves the body of the snail host and reenters presumably through the renal aperture, thence reaching the pericardial cavity. The theory that the cercaria of the BRACHYLAEMINAE leaves the body of the snail host and reenters through some external opening in order to reach its specific destination, such as the kidney, pericardial cavity or salivary glands, was first brought out by Sinitsin in 1931. The ability to infect the pericardial cavity of snails, as reported in this paper, by exposing the snails to cercariae, tends to confirm Sinitsin's theory, and indicates that cercariae shed by a snail host may infect either the same or other snails. Interchange of snail hosts is facilitated by rainy periods when land snails are more active. This interchange explains why, under natural conditions, a large number of snails contain only developing or

fully grown adolescariae in the pericardial cavity without evidence of sporocysts. Of 100 snails, *Eulota similaris,* collected in a *Postharmostomum gallinum*-endemic area in the environs of Honolulu, 95 showed developing or fully grown adolescariae in the pericardial cavity and only 2 showed branched sporocysts in the liver tissue with a few adolescariae in the pericardial cavity; the total number of developing and mature adolescariae counted in each of 20 snails varied from 4 to 28.

That self-infection of a snail with cercariae may also take place was shown by placing a laboratory-raised snail experimentally infected with eggs of *Postharmostomum gallinum* in a jar by itself for a period of about 3 months. After that period the snail was dissected and was found to harbor sporocysts in the liver tissue and adolescariae in the pericardial cavity.

Cercariae shed by a snail, *Eulota similaris,* may enter the body of other species of land snails, *Subulina octona,* and develop into adolescariae. Of 100 *Subulina octona* collected in the same locality as the above-mentioned 100 *Eulota similaris,* 80 were found to harbor in the pericardial cavities from one to five adolescariae which were morphologically identical with those of *Postharmostomum gallinum;* none of them showed the sporocyst stage. There is a possibility that the sporocyst stage does not develop in *Subulina octona.* None of the snails of this species fed eggs of *Postharmostomum gallinum* showed fluke infection when dissected 20 to 40 days later. No attempt was made to expose *Subulina octona* to the cercariae of this fluke.

Postharmostomum gallinum appears then to require two intermediate hosts in its development. The sporocyst-to-cercaria stage develops in one specific snail, *Eulota similaris,* whereas the adolescarial stage may develop either in this snail or in *Subulina octona.* According to Ozaki's findings, *Euhadra peliomphala* and *Phylomycus bilineatus* also serve as secondary intermediate hosts, but whether or not they serve as first intermediate hosts is not known.

SUMMARY

The complete life cycle of *Postharmostomum gallinum* in the intermediate and final hosts has been ascertained. Eggs of these parasites when oviposited contain fully developed miracidia. When ingested by *Eulota similaris,* the eggs hatch and each miracidium may eventually develop in the liver into a branched sporocyst. About 60 days following the original infection, apparently mature cercariae have been found within the sporocyst. Mature cercariae leave the sporocyst and body of the snail host and may reenter the same snail host and reach the pericardial cavity or may infect other snails with which they come in contact, of the same or another species. In order to reach the pericardial cavity, it is believed

that the cercariae enter the renal aperture which opens at the base of the pallial cavity; from this aperture the cercariae enter the kidney cavity and finally, presumably, by crawling into the renal-pericardial aperture, reach the pericardial cavity where they may develop to the adolescarial stage in about 25 days.

Subulina octona harbors adolescariae of *Postharmostomum gallinum,* but this species did not become infected experimentally by feeding them eggs of the fluke.

Chicks 6 to 8 weeks old become infected with *Postharmostomum gallinum* when fed adolescariae from experimentally and naturally infected snails, *Eulota similaris,* and naturally infected *Subulina octona.* These flukes develop to maturity in slightly less than a month.

Bibliography

Alicata, J. E. 1938 The land snail, an intermediate host of the cecal fluke of poultry. Science **88**: 129.

———— 1938 Life history of the cecal fluke, *Postharmostomum gallinum.* J. Parasitol. **24**: 29.

Fukui and Ogata. 1938 Trematode parasites of Jehol. Rep. from Sci. Exp. to Manchoukuo, Sec. 5, div. 1, pt. 1, art. 1.

Guberlet, J. E. 1928 Parasitic worms of Hawaiian chickens with a description of a new trematode. Tr. Am. Micr. Soc. **47**: 444–451.

Sinitsin, D. 1931 Studien ueber die Phylogenie der Trematoden, V. Revision of Harmostominae in the light of new facts from their morphology and life history. Z. Parasitenk. **3**: 786–835.

Explanation of Plates

All drawings made by use of the camera lucida

Plate I

Fig. 1. Fully developed egg enclosing miracidium.

Figs. 2, 3. Sporocysts recovered from a snail 20 days following experimental infection.

Figs. 4, 5. Sporocysts recovered from a snail 40 and 60 days, respectively, following experimental infection.

Figs. 6, 7, 8, and 9. Certain stages of development of germ balls and cercariae in the sporocyst. Lateral view.

Plate II

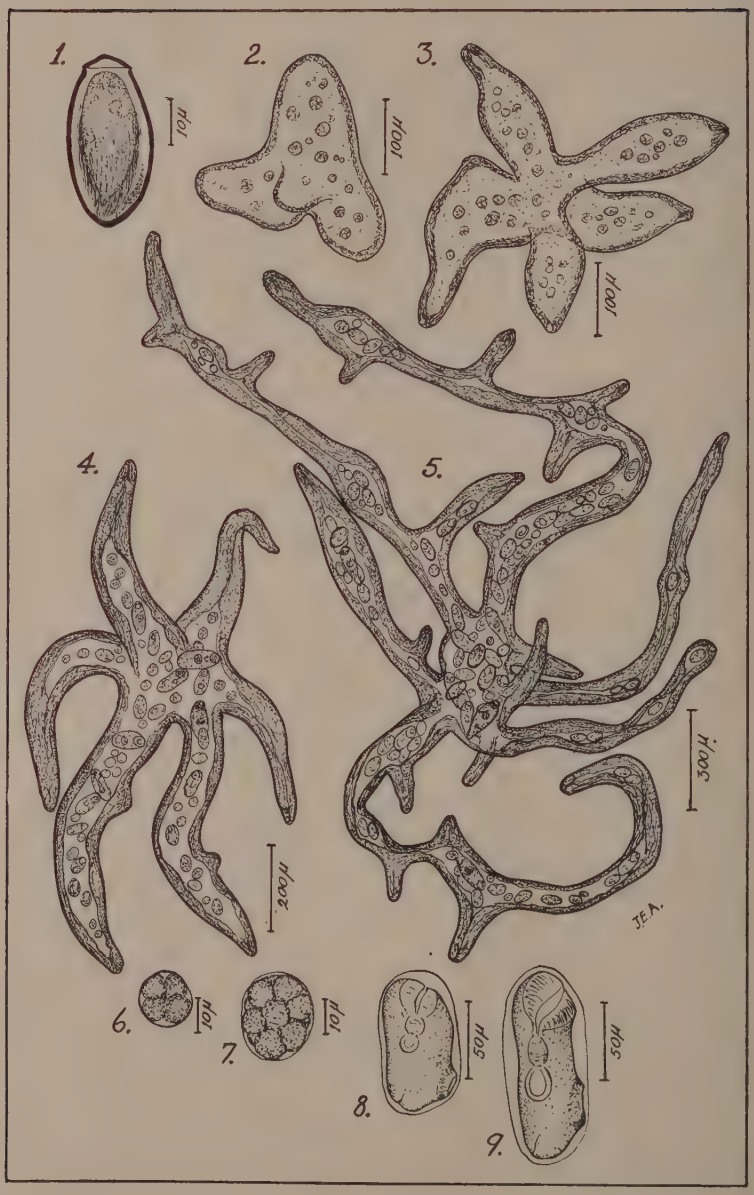
Fig. 10. Mature cercaria showing natural, contracted appearance when inactive in water outside of the snail host. Anterior end bent ventrad. Ventral view.

Fig. 11. Mature cercaria. Ventral view.

Fig. 12. Posterior portion of mature cercaria showing excretory bladder and the 2 lateral excretory ducts emerging from it. Ventral view.

Figs. 13, 14. Developing adolescariae recovered from pericardial cavity of *Eulota similaris* 12 and 25 days, respectively, following exposure of the snails to cercarial infection.

Fig. 15. Adolescaria recovered from pericardial cavity of naturally infected snail, *Eulota similaris.*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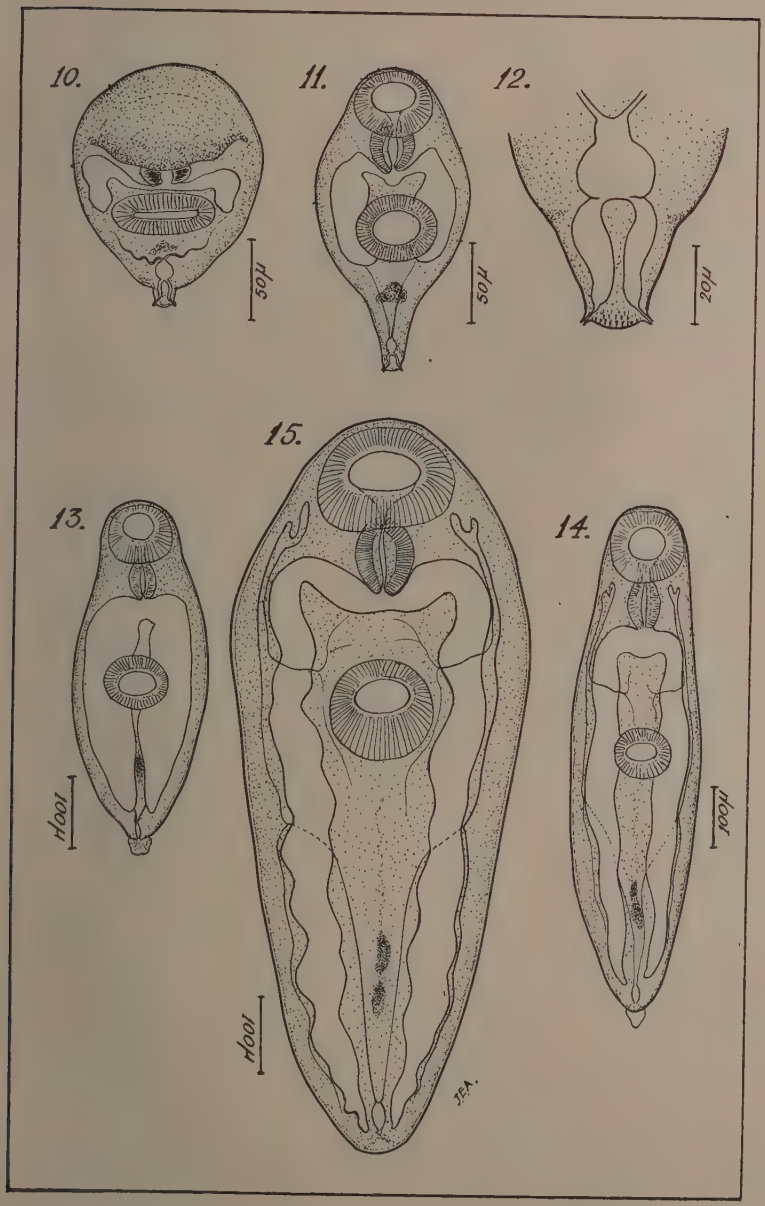
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THE RELATION OF SHADE TO *ANOPHELES QUADRIMACULATUS* BREEDING—A PRELIMINARY REPORT

E. Harold Hinman and Herbert S. Hurlbut*

It has long been recognized that a variety of ecological factors play an important role either in limiting the distribution of anopheline larvae or in regulating their abundance. Temperature, pH, solutes, food, chemical composition of the water, et cetera, may all influence the population density of different species of *Anopheles*. Among other factors light intensity has received considerable study. From a literature review it appears that species respond in different ways to the degree of shade to which they are exposed.

The exact influence of illumination upon anopheline larvae is apparently not understood. Naturally, shade will have a marked effect upon the water temperature. It has also been suggested that since abundant sunlight is essential for photosynthetic activity of the algae, an indirect influence is felt. Likewise, the presence or absence of shade will determine whether aquatic vegetation can grow abundantly and thereby serve as protection from the larvae.

Lamborn (1922) studied changes induced in shaded pools when sunlight was admitted. Very minor chemical changes occurred, although the dominant *Anopheles umbrosus* (malaria-carrying) was replaced by the harmless *A. barbirostris* after clearing of the jungle. Russell (1936) points out that in Assam plants like tarapat, duranta, hibiscus, and wild fig, have been planted along the banks of streams, and where dense shade is produced, *A. minimus* disappears. In a few instances this method has been very effective, but the cultivation of the plants requires close supervision.

Kirk (1937) states that the planting of a belt of shade trees on each side of rivers and streams in Mauritius to impede erosion has prevented the breeding of *A. costalis* (*gambiae*). Light shade is almost as effective a deterrent to oviposition as is dense shade. *Eugenia jambos* is the most suitable shade tree. It is believed that the inhibitory action is through the inability of the necessary food organisms for the larvae to develop in the absence of light. However, *A. funestus* prefers dense shade, but is not an important local transmitter of malaria.

Carr (1938) has experimented with the possibility of limiting *A. albimanus* breeding by shading of streams. He states that natural bodies

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of water which are well-shaded, and which are provided with predacious fish are unfavorable for mosquito breeding. This is due to the fact that horizontal and other vegetation does not flourish under these conditions, and therefore the larvae have no protection against their natural enemies. He utilized *Ficus benjamina* for the production of shade. He also produced artificial shade by constructing a palm leaf thatch roof over a section of a small stream. Prolific *albimanus* breeding was present in floating algae at the initiation of the experiment. After the roof had been in place for one month, the filamentous algae and anopheline larvae had disappeared from the shaded portion of the stream.

Studies of the influence of shade upon breeding of *Anopheles quadrimaculatus* in the Tennessee Valley have been conducted along a number of lines as indicated below.

EXPERIMENTAL PLANTINGS

In view of the world-wide interest in the influence of shade upon anopheline breeding, the Health and Safety Department decided to initiate experimental plantings in various reservoirs of the Authority. Since there are two species of "aquatic" trees native to this area: the bald cypress, *Taxodium distichum,* and tupelo gum, *Nyssa aquatica,* these have been most widely used. The first plantings were begun in the fall of 1935, and throughout the ensuing winter. Tupelo gum and cypress trees, of from two to several years of age, were transplanted from the Pickwick and Wheeler Reservoirs into the experimental area at Jackson Highway Bridge over Shoal Creek, Lake Wilson Reservoir. An area of 3.96 acres with a shoreline of 0.94 mile was planted, all trees being placed in water of one foot or less in depth. Since the original plantings, these areas have received relatively little care.

In the early spring of 1937, 12.5 acres were planted in three different areas in the Pickwick Reservoir. One-year old seedlings of cypress were utilized. Since pond or marsh pine (*Pinus serotina*) grows in standing water in Southern Alabama and Western Florida, two plantings totalling 1.9 acres were made.

With the exception of the pond pine, nearly all of which died from a submergence of water four or more feet deep during the spring floods, there has been a high percentage of survival of these plantings. A satisfactory growth has also been observed to occur in most of the plots. The areas stocked with seedlings have been much more satisfactory to date than those in which transplanted older stock was utilized. The seedling cypress showed a growth increment of approximately twelve inches in the first year.

According to records by Doctor William T. Penfound, Botanist of the Malaria Studies and Control Division, 96 per cent of the tupelo gum

transplanted had survived, and somewhat over 80 per cent of the bald cypress. Of the seedling bald cypress, 95 per cent had survived.

UNCLEARED AREAS

In addition to the experimental plantings, discussed above, three wooded areas were left uncleared within the Wheeler Reservoir which would be subsequently inundated. This was done upon the recommendation of the Board of Malaria Consultants of the Authority and with the approval of the Alabama State Department of Public Health. The Buckeye Swamp area of upland timber comprises 9.6 acres of various kinds of oak, maple, hackberry, elm, tupelo gum, sweet gum and willow. The dead trees, fallen timber, underbrush, and saplings three inches or less in diameter, were removed from the site. This area is practically all located within the top foot of the maximum elevation of the Wheeler Reservoir. Blackwell Swamp is an area of 21.2 acres of tupelo gum trees left uncleared within the upper four feet of the maximum contour. At Harris Station a stand of 1½ acres of willow trees close to the maximum contour was also left uncleared.

Within the Pickwick Reservoir several areas of cypress were left in Collier's Slough. About 29.6 acres of scattered cypress of all sizes at random spacing, were left in the upper five feet of the reservoir. Also, an area of 6.8 acres of small sound cypress of varying size, at approximately 10 foot spacing, was left in the upper five foot zone of the reservoir.

In the Guntersville Reservoir an experimental plot of 27.5 acres was left near Stephenson, Alabama. This contains timber of the following species: water oak, red oak, black gum, American elm, green ash, red maple, and a few hackberries. This area has been subject to occasional flooding in the past, and when the reservoir is impounded none of these trees will be in water exceeding two feet in depth. At least two other smaller areas of dense timber will be left in this reservoir. These areas have been set aside for observation over a period of years. The ability of large trees to withstand partial submergence for at least a portion of the year will be tested. Furthermore, an opportunity will be provided to observe the breeding of *A. quadrimaculatus* under more or less normal conditions in such areas. The results of investigations in certain of these areas during the past are recorded in Table 1, and will be subsequently discussed.

OBSERVATIONS ON ANOPHELINE BREEDING IN SHADED AREAS

During the season of 1937 a number of areas were visited at intervals of one month to determine the effect of shade upon anopheline breeding. In addition to information describing the area, certain physical factors

concerning the habitat were recorded. The light reading was made using a Bewi exposure meter. A reading was made in the shaded area, and then immediately in the open. By a direct conversion the relative intensity of light in the shaded area to that in the direct sunlight (at the time) was measured. Numbers of larvae, per twenty dips in the shaded area, were recorded, together with the water temperature, pH, abundance of flotage, type of vegetation, et cetera.

During the season of 1938 a Weston foot-candle meter (model 614) was used to measure the illumination. The readings are made in foot-candles, based on the International candle, on the surface of the light target. The instrument employs a photo-electric cell. The meter is

FIG. 1. Tupelo gum swamp, Byrd Spring Rod and Gun Club, Huntsville, Alabama.

equipped with three scales reading from 0 to 60, 0 to 120, and 0 to 600. The lowest scale is graded in individual foot-candles, and readings to the half foot-candle may be made readily. Several readings were taken in each of the areas and the average recorded, in addition to the minimum and maximum. An attempt was made to visit each area twice monthly during the breeding season. Observations were made only on sunny days, and an attempt was made to secure the readings between 10:00 AM and 3:00 PM in order to obtain the maximum illumination present in the area.

The areas selected for study were those providing the densest shade. The illumination varied considerably, owing to the irregular distribution

of the trees. In many instances, small light flecks could filter through to the water surface. At such points the illumination would, of course, measure in excess of 600 foot-candles, and these flecks were disregarded. They are not included under the average given in the table.

Blackwell Swamp, Buckeye Swamp, and Collier's Slough, have been described. The Byrd Spring area comprises approximately 600 acres of dense tupelo gum (*Nyssa aquatica*). The trees average in excess of 100 feet in height. The denseness of the stand is emphasized by the small degree of underbrush and herbaceous vegetation present in the area. (See Figs. 1 and 2.) The water elevation is controlled by means of

FIG. 2. Interior of tupelo gum swamp, Byrd Spring Rod and Gun Club, Huntsville, Alabama.

a dam and varies in the areas studied from 1 to 24 inches in depth. Long Pond and Little Egypt Pond are limesinks which had their elevation raised considerably by the impounding of Wheeler Reservoir, and thus flooded out into an area of upland timber comprising willow oak (*Quercus phellos*), hackberry (*Celtis* sp.), overcup oak (*Quercus lyrata*), red gum (*Liquidambar styraciflua*), water oak (*Quercus laurifolia*), rough leaf dogwood (*Svida aspenfolia*), hawthorne (*Crataegus* sp.), et cetera. Meadow Pond is an old limesink with a marginal fringe of willows, buttonball and a few ash, honey locust, and hawthorne. Many of the buttonball bushes had a heavy undergrowth of hemp vine (*Mikania scandens*), and thereby presented a rather dense shade.

Table 1 summarizes the light readings, water temperature, pH of the water, and the abundance and species of anopheline larvae in these areas. Since only mature larvae can be identified with accuracy and the rearing out of immature ones entails considerable difficulty, the number of larvae identified appear to be relatively few. Records in this table are included only for June, July, and August, 1938, the season when maximum shade occurs. *Anopheles quadrimaculatus* has been present in every one of the shade areas investigated. Uniformity of illumination does not exist in the wooded areas studied. Considerable range in light intensity existed within the individual area as shown on Table 1. Invariably one could locate isolated patches where enough light filtered through the vegetation so that the intensity exceeded 600 foot-candles—the maximum our instrument would record. Stations were therefore located in the more densely shaded areas. The distribution of *A. quadrimaculatus* within any given shaded area has shown no correlation with light intensity. The larvae were found at the maximum and also at the minimum readings. In attempting to correlate the relative abundance of this species of larva with the average low light readings again no correlation is present. Meadow Pond No. 7 with a higher illumination had a significant number of *quadrimaculatus* larvae, Meadow Pond No. 15 with a moderate light reading had heavy breeding, and Long Pond No. 2 with a low light intensity also had abundant breeding of this species.

Table 1 includes two unshaded areas in adjoining parts of Byrd Spring and Long Ponds. Obviously there could not be any profound difference in the chemical composition of the water of the unshaded and shaded areas. In the case of the Long Pond stations, they are separated by 20 feet. To obviate the possibility of larvae drifting into a shaded area from an unshaded one, a burlap fence was established about one naturally shaded area (see discussion of section on Experimental Shade). Opportunity was afforded to study certain areas (Byrd Spring No. 1 and Meadow Pond No. 7) which might be classified as intermediate between dense shade and absence of shade. *A. quadrimaculatus* breeds in such situations as well as in open water and in dense shade.

TABLE 1.—*Shade study. Tabulations of data for months of June, July and August, 1938*

Location	Vegetation giving shade	Light readings in foot-candles				°C temp. at surface		pH at surface	Specimens of *Anopheles* larvae			Number of inspections	Total larvae	Ave. larvae per 20 dips
		Low readings		Modal readings										
		Range	Ave.	Range	Ave.	Range	Ave.	Range	*cruc.*	*punc.*	*quad.*			
Blackwell	Tupelo Gum	40–110	92	120–160	147	21–25	24	6.6–6.8	2	0	10	6	17	3
Buckeye No. 1	Willow oak and Slippery elm	130–180	148	200–250	215	23–27	25	6.0–6.6	4	1	4	5	34	7
Buckeye No. 2	Lizard's tail	70–130	97	100–200	157	26–27	26	6.2–6.4	1	0	1	3	26	9
Byrd Sp. No. 1	Giant smartweed	170–350	253	350–600	458	24–28	26	6.6–7.4	16	5	12	6	145	24
Byrd Sp. No. 2	Tupelo Gum	40–70	54	70–150	114	22–25	23	7.0–7.6	31	58	28	6	125	21
Byrd Sp. No. 3	Unshaded area in tupelo swamp	.....	..	.....	...	25–29	28	6.6–6.8	10	3	14	4	158	39
Long Pond No. 1	Mostly unshaded 20 feet from No. 2	.....	..	.....	...	28–30	29	7.5–8.0	0	0	30	4	105	26
Long Pond No. 2	Hawthorn, willow oak, sweet gum and supple jack	15–40	24	40–80	57	26–30	28	7.2–8.0	1	1	56	6	133	22
Meadow Pond No. 7	Black willow, honey locust, hawthorn and willow oak	140–310	242	250–450	354	24–32	29	6.2–6.6	3	0	6	6	73	12
Meadow Pond No. 15	Buttonball bush and hempvine	50–270	125	150–550	319	22–31	28	6.2–6.6	35	1	15	6	559	93
Collier's Slough No. 2	Bald cypress	140–260	185	210–350	285	27–30	28	7.2–7.8	0	0	0	6	0	0
Collier's Slough No. 3	Bald cypress	60–150	106	180–240	206	28–30	29	6.8–7.6	0	3	12	6	91	15
Totals									103	72	188	64	1466	

It is interesting to compare the distribution of the three species of anophelines according to light intensity. Our primary interest was in *Anopheles quadrimaculatus,* but data were secured on *punctipennis* and *crucians*. With the exception of Byrd Spring Station No. 2, which had rather dense shade, *A. punctipennis* was relatively scarce or absent. In this particular situation it was the most abundant anopheline. This area presented the lowest average water temperature encountered in the study during June, July, and August.

Despite the fact that in our routine malaria control work *Anopheles crucians* is rarely encountered, larvae were, with one exception, found in all these shade stations containing *A. quadrimaculatus*. The distribution of this species has been studied in some detail in Meadow Pond during the past two years. In the unshaded water it is rarely secured but has been the dominant species in certain of the shade stations (Nos. 15, 17, 19). It is noteworthy that the pH of these areas was always below 6.7.

A number of other shade stations have been under investigation both during 1937 and 1938. Since the light intensity was recorded by a Bewi exposuremeter during 1937, these results are not directly comparable and have been omitted from this report. The findings, however, checked with those listed in Table 1. Certain stations visited during part of the season of 1938 have likewise been omitted since it is felt that the breeding of *A. quadrimaculatus* throughout the entire season in shaded areas is of greater significance than the occasional finding of them.

STUDIES DURING 1939

Routine observations upon most of the areas included in Table 1 have been continued at bimonthly intervals during the summer of 1939. The occurrence of *Anopheles quadrimaculatus* and *A. crucians* were in approximately the same proportion as previously observed. *A. punctipennis* was found in two additional shaded areas not previously reported —namely Buckeye and Blackwell Swamp. The light readings, water temperatures and pH range were not significantly changed over those of 1938.

EXPERIMENTAL SHADE

An area in Long Pond which was breeding *Anopheles quadrimaculatus* in moderate abundance was selected. A ridge pole was constructed, and a tarpaulin 28 feet by 20 feet was suspended across the ridge pole and supported by ropes to nearby trees or shrubs on August 5, 1938. It was hoped to thereby obtain information on the relative attractiveness or repellant effect of dense shade to *A. quadrimaculatus*. The heavy tarpaulin prevented the penetration of light and since it was supported from one to three feet above the surface of the water, little direct sunlight was able to enter about the periphery of the tent. In order to eliminate the

possibility of larvae or ova drifting into or out of the area, a barrier of burlap material was established about the edge, but placed somewhat within the periphery to exclude the marginal area exposed to higher illumination. The burlap was attached to stakes driven in the ground, was 20 inches in width, approximately half of which projected above the surface of the water and half below. Inspections of breeding were made twice weekly and recorded in terms of numbers of larvae per twenty dips. The light readings were made less frequently, and were staggered at different times of the day in order to detect the possibility of light filtering under the tent at either the early morning hours or late afternoon. The tarpaulin was removed September 16 to determine the resultant effect.

Table 2 and Fig. 3 summarize the results of this experiment. The continued presence of first stage larvae was taken as evidence of oviposition within the shaded area.

TABLE 2.—*Larval counts for area artificially shaded by tarpaulin.*

Date of inspection	Shaded area					Control area				
	No. larvae per 20 dips	Instars*				No. larvae per 20 dips	Instars*			
		1	2	3	4		1	2	3	4
Aug. 9	15	8	5	0	2	31	7	18	4	2
Aug. 11	15	3	3	5	4	19	8	6	4	1
Aug. 15	16	5	4	3	4	36	12	13	7	4
Aug. 19	9	0	2	3	4	37	1	13	11	12
Aug. 22	16	0	10	6	0	47	11	13	11	12
Aug. 27	13	1	0	8	4	32	4	19	8	1
Aug. 29	7	3	1	3	0	33	14	6	8	5
Sept. 2	14	2	0	12	0	46	20	10	12	4
Sept. 6	15	8	1	4	2	36	11	8	8	9
Sept. 9	8	1	7	0	0	41	9	18	8	6
Sept. 13	2	2	0	0	0	37	11	11	9	6
Sept. 16	3	1	1	1	0	49	5	17	18	9
Totals	133	34	34	45	20	444	113	152	108	71

* All larvae identified were *A. quadrimaculatus.*

The range of light intensity as measured along the north-south axis of the area was 0.5 to 6.0 foot-candles with an average of 2.9 foot-candles between 8:00 AM and 3:00 PM. Portions of the periphery of the area were found to be exposed to direct sunlight for brief periods before 9:30 AM or after 3:00 PM. Readings in excess of 600 foot-candles were encountered upon occasion at these times of the day at the margin, but during the middle portion of the day all direct sunlight was excluded and reflected light of low intensity prevailed. The average of the light intensity at the periphery was 12.5 foot-candles. The average intensity of the entire area at different periods of the day was 7.7 foot-candles.

The results listed in this table indicated that *A. quadrimaculatus* would continue to oviposit in relatively dense shade (averaging 7.7 foot-candles), that the ova hatch, and that the larvae appear to develop normally under these conditions. It is true that the intensity of the breeding diminished during the course of the experiment.

Before erection of the tarpaulin the breeding in this area varied from 59 to 19 larvae per 20 dips. After the shade was provided, it varied from 16 to 2 larvae per 20 dips. In the control area it is interesting to note that a marked increase occurred about September 19 and that an increase also occurred in the experimental area following removal of the tarpaulin on September 16. It is probable that this latter increase should not be attributed exclusively to the removal of the shade, but also in part to the

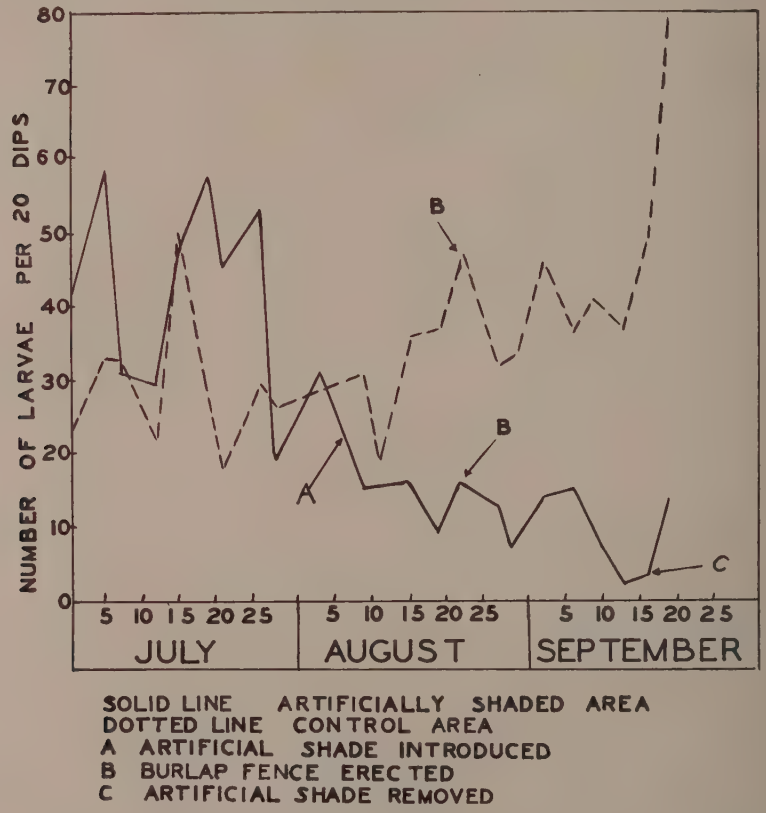

FIG. 3. Larval density in artificially shaded area before and after introduction of shade.

natural increase in breeding. Drainage of the area prevented continuation of the observations beyond September 28.

It has always been assumed that oviposition occurs in the majority of instances at night, and therefore, that shade itself would probably have little effect in the selection of the places for egg deposition. The development of the larvae in this situation would seem to indicate that none of the elements essential for normal growth were lacking.

THE REARING OF *Anopheles quadrimaculatus* IN THE COMPLETE ABSENCE OF LIGHT

An attempt was made to rear *A. quadrimaculatus* in the complete absence of light. Fertilized female *quadrimaculatus*, from our insectary strain, were placed in a small incubator room in the basement of our laboratories. The incubator is well-sealed and held at a constant temperature of 20° C. After oviposition the ova were placed in small rearing pans and the larvae fed in a manner similar to that utilized in the insectary rearing of the species. No light was permitted in the room, except a small flashlight which was used for the briefest duration possible in making the necessary manipulations. It was not always necessary to care for the strain daily. When pupae were secured, arrangements were made to liberate the adults into a cylindrical cage constructed of mosquito netting six feet in height and fourteen inches in diameter, constructed with a small sleeve for the feeding of adults. It appeared to the observers that the larvae developed normally under these conditions, and at a rate quite comparable to what might be expected considering the low temperature, to that of the development of larvae in the open. The majority required from 30 to 35 days from the date of oviposition till the emergence of adults. Our insectary strain, at approximately 24° C, requires 15 to 20 days. About 300 adults were liberated in this cage, were offered dextrose and human blood as food. The females fed satisfactorily on the blood of the attendant which was supplied daily.

Although adults survived in excess of 60 days, no ova were ever secured. Dissection of a few of the females indicated that fertilization had not occurred, although normal ovarian development was found. A second attempt to rear a strain of *Anopheles quadrimaculatus* under similar conditions has been made. Ova were obtained from our insectary colony and the larvae reared as before. After 1400 adults from this rearing had been liberated in the cage, no oviposition had occurred. The cage was therefore removed to the insectary for one night where the temperature ranges between 21° C and 24° C. It was thought that conditions in the insectary might be more favorable for mating. The cage was removed before daylight and returned to the incubator room. Four days later about 300 eggs were deposited, 91 per cent of which subsequently proved to be fertile. The results of this experiment seem to indicate that light *per se* is not essential for this species to complete its life cycle.

SUMMARY

An investigation of the influence of shade upon the breeding of *Anopheles quadrimaculatus* has been undertaken along five different lines. Experimental tree plantings in reservoirs of bald cypress and tupelo gum have been made, but many years must elapse before any definite conclu-

sions can be drawn. Pond pine failed to survive the conditions to which the species was subjected.

Wooded areas were left uncleared below the high water contour. *A. quadrimaculatus* has bred in these following impounding of the reservoir.

Known shaded breeding grounds of the species have been visited at regular intervals and the illumination, pH, water temperature, and abundance of breeding recorded. It has not been possible to establish a definite correlation between intensity of illumination and the abundance of breeding. It has been observed that in some of the more densely shaded areas the intensity of breeding was less than that encountered in waters exposed to direct sunlight, well overgrown with aquatic vegetation. The optimum conditions for breeding of this species appear to be clean, open water with dense aquatic vegetation or abundant flotage. Natural shade restricts the vegetation and thereby decreases the protection and food of the larvae, thus indirectly influencing breeding. No evidence of shade preventing the occurrence of breeding has been encountered.

Artificial shade was established by supporting a tarpaulin over an area of natural breeding of *A. quadrimaculatus*. Breeding of the species continued, as indicated by the presence of first instar larvae, though considerably diminished, for the period of six weeks during which shade was maintained.

Anopheles quadrimaculatus has been reared through its entire life cycle in an incubator room at 20° C in the complete absence of light.

Bibliography

Carr, H. P. 1938 *A. albimanus* Breeding in Relation to Degree of Shade in Breeding Places. South. Med. J. **31**: 803–805.

Kirk, J. B. 1937 The Relation of Shade to the Natural Breeding Places of *Anopheles costalis* in Mauritius. Ann. Trop. Med. and Parasitol. **31**: 1–6.

Lamborn, W. A. 1922 Some Problems of the Breeding Places of the Anophelines of Malaya; a Contribution Towards Their Solution. Bull. Entom. Research **13**: 1–23.

Russell, P. F. 1936 Malaria in India. Impressions From a Tour. Am. J. Trop. Med. **16**: 653–664.

RESEARCH NOTES

TRICHINOSIS IN AN AMERICAN BADGER, *TAXIDEA TAXUS TAXUS*

On September 1, 1939, an autopsy was performed on a female American badger (*Taxidea taxus taxus*) from the collection of the New York Zoological Park. The autopsy revealed the entire musculature peppered with white, pin-point flecks which were most numerous in the diaphragm, intercostal, psoas and tongue muscles. Microscopic examination of muscle tissue showed a very extensive infection with *Trichinella spiralis*. The grossly visible evidence was caused by advanced stages of calcification of these nematode larvae. No other pathology was present.

The badger was originally captured in Wyoming in 1936 at about six months of age. Previous to her arrival in the Park collection, for approximately eight months she was fed a cereal diet. The following five months her diet consisted mostly of cream cheese, liverwurst and cooked meat. For the two years while the animal was in the Park her diet consisted of raw beef, vegetables and fruits. The question naturally arises whether the liverwurst was the source of infection.

The animal had a history of going off feed once or twice a year for from one to three days. For the three weeks immediately preceding her death her appetite had been inconstant, with no other discernible symptoms.

It is interesting to recall that Herbst in 1851 (Nachr. K. Gesellsch. Wissensch. Georg-Aug. Univ. Göttingen **19** (Dec. 29): 260–264) used muscles from a naturally infected European badger (*Meles meles*) in some of the earliest successful transmission experiments with *Trichinella*. This nematode has been reported from many North American carnivorous animals and although it has been reported frequently from the European badger, the authors believe this is the first record of infection in the American badger. This adds one more wild species to the list of potential reservoir hosts of a parasite of man.—Carlton M. Herman and Leonard J. Goss, *Hospital and Laboratory, New York Zoological Park.*

AN *ENDAMOEBA* PARASITIC IN *OPALINA CHOROPHILI*

In recent years several papers have been published describing parasitic amoebae within the bodies of certain opalinid ciliates which in turn are parasitic in the recta of certain amphibia. This seems to be a case of true hyperparasitism.

Carini (1933a, Ann. Parasitol. **11**: 297; and 1933b, Arch. Biol. **16**: 80) recognized these organisms as parasites. He named two species *Brumptina brasiliensis* and *Brumptina paulista*. Their identity as endamoebae was established by Stabler (1933, J. Parasitol. **20**: 122), and confirmed by Carini and Reichenow (1935, Arch. Protistenk. **84**: 175–185). Since the name *Endamoeba brasiliensis* has been previously given to an endamoeba from man, Stabler and Chen (1936, Biol. Bull. **70**: 56–71) suggest that this organism should be named *Endamoeba paulista* (Carini, 1933) if it is a distinct species.

Chen and Stabler (1936, Biol. Bull. **70**: 72–77) have reported all four genera of the family Opalinidae parasitized by the endamoebae. The parasites have a very wide geographical distribution. Chen and Stabler (1936) give records from Egypt, China, Ceylon, the United States, and South America. Their records for the United States are from Utah and Arizona. These involve three species: *Zelleriella hirsuta* from *Bufo cognatus;* and *Opalina woodhousii* and *Zelleriella* sp. from *Bufo woodhousii*.

In my studies on the ecology and life history of opalinids I have found these "hyperparasitic" amoebae only once. This was in the case of *Opalina chorophili* Metcalf, 1923 from the swamp cricket frog *Pseudacris triseriata* (Wied, 1838), collected near Wilmington, Ohio in March, 1937. About 50 per cent of the frogs contained parasitized opalinids. From 1 per cent to 95 per cent of the opalinids in any given frog were parasitized and these were chiefly the adults.

In the living opalinids the endamoebae when inactive might be confused with nuclei, but they are much larger and frequently more numerous. Infections of

endamoebae vary in number from less than ten to well over one hundred individuals for each ciliate. Oftentimes the endamoebae are all in the same stage of development, but occasionally inactive trophozoites, cysts, and active trophozoites may be observed within a single opalinid. Movements of active trophozoites within the cytoplasm of the ciliate are common.

The trophozoites vary in length from 11 to 14 microns, and contain one nucleus. In the cytoplasm a few small refractile granules occur. The spherical inactive trophozoites have an average diameter of 12 microns.

The uninucleate cysts average about 9 microns in diameter. The cytoplasm appears as a somewhat grayish homogeneous substance.

Whether a harmful effect is produced by the endamoebae is not known; however, living opalinas containing heavy infections showed disintegration of the cell membrane more quickly and rapidly than non-infected individuals. When the cell membrane ruptures the endamoebae whether active or encysted flow out into the surrounding medium. In one instance, after escaping from the body of the ciliate, the trophozoites continued to move about on the slide for 10 minutes.

The problem of how the endamoebae gain entrance into their astomatous hosts in the first place is a rather puzzling one. So far as I know this process has never been observed.—F. O. HAZARD, *Department of Biology, Wilmington College, Wilmington, Ohio.*

CURTAILMENT OF EGG PRODUCTION IN THE COMMON RING-NECKED PHEASANT DUE TO EXPERIMENTAL INFECTION WITH THE OVIDUCT FLUKE, *PROSTHOGONIMUS MACRORCHIS*

It has been recently reported by Gower (1939, J. Parasitol. **25**: 447–448) that the common ring-necked pheasant, *Phasianus colchicus torquatus,* is highly refractive to the oviduct fluke *Prosthogonimus macrorchis* Macy. Gower's experiments, as he pointed out, were quite limited, involving but three birds. His results do not agree with recent work I have completed.

With facilities furnished by the Division of Entomology and Economic Zoology of the University of Minnesota, under the direction of Dr. William A. Riley, and with the aid of a grant furnished by the Minnesota Academy of Science, the writer infected four laying pheasants with cysts of *Prosthogonimus* from 40 dragonfly naiads of the genus *Tetragoneuria,* May 8, 1939. On June 4, 250 large specimens of *Prosthogonimus macrorchis* were recovered from the oviducts of these birds. During this period a control group of four birds laid five times as many eggs as the infected birds, and the latter group exhibited typical symptoms of such infection. It was thus shown that ring-necked pheasants are fully as susceptible to infection as the domestic hen, and that egg laying is greatly reduced by the presence in the oviducts of *Prosthogonimus macrorchis.*

A full report on these experiments is being delayed pending more information regarding natural infection of these birds by *Prosthogonimus macrorchis.*—RALPH W. MACY, *College of St. Thomas, St. Paul, Minnesota.*

SOME BLOOD PARASITES FROM MONTANA BIRDS

For the most part studies dealing with the blood parasites of North American wild birds have been confined to the northeastern and midwestern states. The present study is concerned with material collected by the junior author in Beaverhead County, Montana, during 1937. Blood smears were taken from twenty-two birds of four species and the parasites found are tabulated in Table 1.

The *Haemoproteus* and *Leucocytozoon* parasites from Swainson's hawk are apparently new host records. The Haemosporidia found in the magpie and the crow have been recorded from these hosts before but in other localities.

Descriptions of the above protozoan parasites, with the exception of the *Haemoproteus* from Swainson's hawk, together with their host records were given

TABLE 1. *Blood Parasites Found in Montana Birds*

Common name	Scientific name	Age	Number		*Haemoproteus*	*Leucocytozoon*	*Plasmodium*	Microfilaria
			Examined	Infected				
Magpie	*Pica p. hudsonia*	adult	7	4	*H. picae* (3)	*L. berestneffi* (2)	*P.* sp. (1)	microfilaria (2)
"	"	immat.	2	1	*H. picae* (1)			
"	"	fledg.	6	0				
Crow	*Corvus b. brachyrhynchos*	adult	1	1	*H. danilewskii* (1)			
"	"	immat.	3	1		*L. berestneffi* (1)		
Swainson's hawk	*Buteo swainsoni*	adult	2	2	*H.* sp. (1)	*L. mathisi* var. *buteonis* (1)		
Great horned owl	*Bubo v. pallescens*	immat.	1	0				
Totals			22	9	6	4	1	2

The figure in parentheses denotes the number of birds harboring the particular parasite.

by Coatney and Roudabush (1937, Am. Midland Naturalist **18**: 1005–1030), and Coatney and West (1938, *ibid.*, **19**: 601–602).

The microfilariae found in the magpies were of two distinct forms and were present in about equal numbers. The smaller form averaged about 71 μ long and 5.6 μ wide. The larger forms averaged 138 μ long and 7 μ wide. Though slightly larger, the latter may be identical with the parasites reported from the magpie by Coatney and Roudabush (loc. cit.).—G. Robert Coatney and William L. Jellison, *Division of Infectious Diseases, National Institute of Health, U. S. Public Health Service.*

PHYTOMONAD FLAGELLATES FROM MEXICAN MILKWEEDS

Latex flagellates have been reported from milkweeds and related plants from all over the world since their discovery by Lafont in 1909 on the island of Mauritius. To the writer's knowledge their occurrence in Mexico has not been recorded. During the summer of 1939 while engaged in field work in various parts of Mexico a number of milkweeds were examined for flagellates. As shown in the table three species of plants were examined, all belonging to the genus *Asclepias*. One of these species (*A. glaucesens*) is a new host record for phytomonads. Small drops of

Table 1.—*Phytomonad flagellates in Mexican milkweeds.*

Species of plant examined	Site of collection	No. plants examined	No. plants infected	Percentage of plants infected
Asclepias glaucescens	Cuernavaca, Morelos	100	1	1
Asclepias curassavica	Cuernavaca, Morelos	25	1	4
Asclepias neglecta	Rio Frio, Puebla	100	0	0
Asclepias glaucescens	Cuautla, Morelos	54	0	0
Asclepias curassavica	Cuautla, Morelos	39	11	30
Asclepias curassavica	Cozumel, Quintana Roo	171	5	3
Totals		489	18	3.7

latex from two or three different parts of the plant were examined in physiological saline (0.85 per cent) soon after the plants were collected. Examinations were made with the 16 mm objective. Slides stained with Giemsa's blood stain were prepared in some cases when flagellates were found. No attempt has been made to classify the organisms, but from cursory examinations they resemble most closely *Phytomonas elmassiani* (Migone). Data are presented in Table 1.—Reginal Hewitt, *Protozoology Laboratory, Instituto de Salubridad y Enfermedades Tropicales, Mexico, D. F.*

AMERICAN SOCIETY OF PARASITOLOGISTS

FIFTEENTH ANNUAL MEETING, COLUMBUS, OHIO,

DECEMBER 28, 29 AND 30, 1939[1]

Minutes of Fifteenth Annual Business Meeting

The fifteenth annual business meeting of the Society was held in Pomerene Hall, Ohio State University, on December 29, 1939, following the annual Parasitologists' Luncheon, which was attended by 133 members and guests. The meeting was called to order at 1:55 PM by President Horace W. Stunkard.

I. REPORTS OF OFFICERS

1. The report of the Secretary was presented. As of December 20th, there were 421 members[2] on the roll, including 24 in arrears for 1939 dues. Twenty-four new members were added during the course of the year, and, in addition, 24 more new members were elected by the Council on December 27th. The deaths of the following members occurred during the past year:

Edwin C. Linton, vice-president of the Society in 1927, died in Philadelphia, Pa., June 5, 1939.

Arnaldo Giovannola died in Mexico City, October 11, 1939.

Upon motion the report was accepted and placed on file.

2. In the absence of Dr. Otto, the Treasurer's report was presented by the Secretary. For the fiscal year, 1939, there was a favorable balance of $244.80 which reduced the accumulated deficit carried forward from previous years from $481.96 to $237.16. The report of the Treasurer, certified as correct by the Auditing Committee of the Council, was accepted and placed on file.

II. REPORTS OF COMMITTEES

1. The report of the status of the Secretarial Fund was presented by Dr. Norman R. Stoll, Custodian. Upon motion, the report, certified to be correct by the Auditing Committee, was accepted and placed on file.

2. The report of the Editorial Committee was presented by Dr. Norman R. Stoll, Chairman.

> "THE JOURNAL OF PARASITOLOGY itself (Volume 25 in 6 numbers of 536 pages, and the December Supplement of 61 pages), regularly issued on schedule, constitutes the report of the Editorial Committee for 1939."

In a supplementary report, Dr. Stoll presented an analysis showing the proportion of the JOURNAL, by weight, devoted respectively to articles on protozoology, helminthology and medical entomology during the past two years.

3. The report of the Biological Abstracts Committee, Dr. D. H. Wenrich, Chairman, was presented by the Secretary. Following the recommendation of the Council, the following resolution was voted:

> "Resolved that Biological Abstracts could better serve the interests of the members of this Society by the publication of adequate taxonomic abstracts."

Dr. G. W. Hunter, III, called attention to the plan whereby members of a Society, 30 per cent of whose members subscribe to Biological Abstracts, may

[1] Abstracts of papers presented were published before the meetings in THE JOURNAL OF PARASITOLOGY, Dec. Suppl., 1939. A general report of the program appeared in *Science*, Feb. 2, 1940 (91: 112).

[2] A map is presented showing distribution of the 371 members in the United States.

secure a $1.00 reduction in the subscription price to any section or sections of Biological Abstracts. The Secretary was instructed to take steps to find out how many members of the American Society of Parasitologists subscribe to Biological Abstracts, and thus ascertain whether members of the Society could expect to qualify for this benefit.

III. Reports of Representatives of the Society

1. A report of the representatives on the Council of the American Association for the Advancement of Science was presented by Dr. Ernest C. Faust. Upon motion the report was accepted.

2. The report of the representatives on the Council of the Union of American Biological Societies was given by Dr. George L. Graham. An analysis was presented showing the marked reduction in the interval between the publication of articles and the appearance of abstracts in Biological Abstracts. In 1938, as compared with 1928, this interval was reduced an average of 83 per cent for articles published in The Journal of Parasitology, the average interval for 1938 articles being 88 days. Upon motion, the report was accepted.

IV. New Business

A report of the actions of the Council at its meeting held on December 27, 1939, was presented by the Secretary. The following items were noted:

1. The enactment of a set of by-laws (copy appended) to codify existing acts of Council governing procedures of the Society.

2. The proposal of two amendments to the constitution of the Society as follows:

> The Journal of Parasitology. This Journal, the property of the Society, is its official organ. Responsibility for its conduct shall rest with the Council which shall select the editorial staff and set the price of subscription.
>
> Endowment Fund. Provision is made for the establishment of a permanent endowment fund, the principal of which may be expended only by a three-fourths vote of all members of the Council and approval by a three-fourths vote of the members of the Society present at a regular

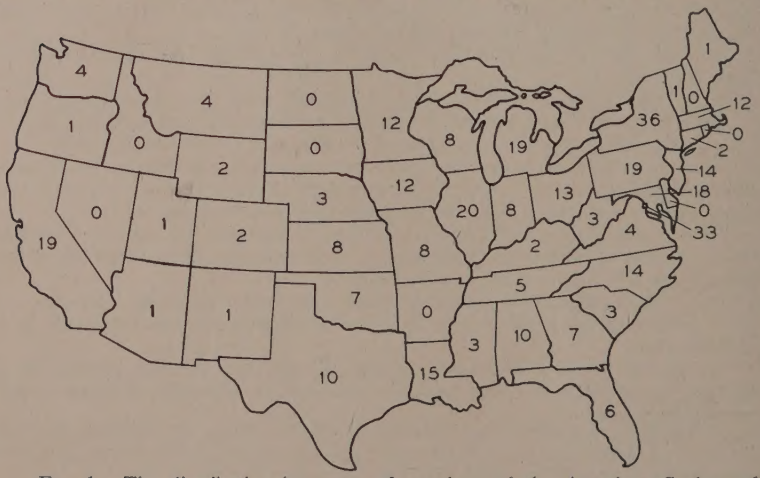

Fig. 1. The distribution by states of members of the American Society of Parasitologists.

meeting. The Council shall be entrusted with the maintenance of the fund, and the use of the income therefrom.

3. The election of Dr. N. H. Swellengrebel of the Colonial Institute, Amsterdam, Holland, as a Foreign Honorary Member of the Society.

4. The approval by Council of a plan to publish an index of the first 25 volumes of THE JOURNAL OF PARASITOLOGY during the coming year. After discussion, it was moved that the Treasurer be authorized to include bills for the index along with the bills for dues to be sent out next fall. Motion seconded and passed.

Upon motion in accordance with the recommendation of the Council, Dr. Charles A. Kofoid was unanimously elected a life member of the Society.

The Secretary presented the Council nominations for officers of the Society for 1940, as follows: President, David H. Wenrich; Vice-president, Gotthold Steiner; Secretary, for two years, Oliver R. McCoy; Members of the Council, for four years (to succeed A. C. Chandler and E. C. O'Roke), Clay G. Huff and Horace W. Stunkard; Members of the Editorial Board, for four years (to succeed E. R. Becker, O. R. McCoy and R. P. Strong), Harold W. Brown, Harold W. Manter and Reginald D. Manwell; Representatives on the Council of the American Association for the Advancement of Science, George R. LaRue and William A. Riley; Representatives on the Council of the Union of American Biological Societies, George L. Graham and Earl C. O'Roke; Representative on the National Research Council, for three years, W. W. Cort. There were no further nominations and upon motion, duly seconded, the Secretary was instructed to cast one ballot for the nominations as presented.

A vote of thanks was extended to the Secretary and to Dr. Carl E. Venard, local representative of the Society in Columbus, for their services in making arrangements for the Columbus meeting.

At 2:45 PM it was voted that the meeting of the Society adjourn to convene again in December, 1940, at Philadelphia, Pa., in conjunction with the meeting of the American Association for the Advancement of Science.

Respectfully submitted,

O. R. McCOY, *Secretary*

AMERICAN SOCIETY OF PARASITOLOGISTS

BY-LAWS

MEMBERSHIP

1. *Election of Members.* An affirmative vote of all Council members present at a meeting shall be necessary for the election of candidates for membership. If the vote is taken by mail ballot, an affirmative vote of all members of the Council replying within thirty days shall be required.

2. *Members in Good Standing.* Members in good standing are those whose current dues are paid.

3. *Delinquent Members.* THE JOURNAL OF PARASITOLOGY shall not be sent to members in arrears, and members in arrears for the current year and two previous years shall be dropped from the roll of the Society at the end of the current year.

4. *Reinstatement of Delinquent Members.* Members dropped for non-payment of dues, or who have resigned, may be reinstated by the payment of all dues in arrears. Otherwise, the applicant must apply for election as a new member.

5. *Non-subscribing Members.* Where a second membership in the Society is taken in the same immediate family, the second member may join upon the payment of annual dues of one dollar, but without receiving THE JOURNAL OF PARASITOLOGY.

6. *Honorary Life Members.* Upon unanimous vote, the Council may recommend that the Society confer honorary life membership upon distinguished American parasitologists over sixty years of age. Honorary Life Members shall enjoy

full membership privileges and shall be exempted from the payment of dues. The number of Honorary Life Members at any one time shall be limited to five.

7. *Foreign Honorary Members.* Upon unanimous vote, the Council at its annual meeting may elect Foreign Honorary Members. No two men from the same country shall be elected in the same year. The number of Foreign Honorary Members at any one time shall be limited to twelve. Foreign Honorary Members may receive THE JOURNAL OF PARASITOLOGY upon payment of membership dues.

PRESENTATION OF PAPERS AT ANNUAL MEETINGS

1. Except for invited papers, and papers coming to the American Society of Parasitologists program through joint sessions with another Society or Section, all persons presenting papers must be members of the Society in good standing, or must be introduced by a member in good standing.

2. Each member of the Society in good standing may be allotted not more than fifteen minutes of program time, to be used in person or by a non-member introduced by him.

3. Papers offered for presentation too late to be included on the printed program may be presented at the conclusion of any of the scientific sessions provided that the presiding officer obtains the consent of the members present.

OFFICERS

1. The Council shall act as a nominating committee and at the annual meeting shall submit to the Society at least one nominee for each office to be filled. The Secretary shall invite the members of the Society to submit nominations for consideration by the Council.

2. The Treasurer of the Society shall act also as Treasurer for THE JOURNAL OF PARASITOLOGY and shall be bonded for the sum of two thousand dollars.

3. A sum of fifty dollars shall be allotted annually to the Secretary, to the Treasurer, and to the Chairman of the Editorial Committee for attendance at the annual meeting.

MANAGEMENT OF THE JOURNAL OF PARASITOLOGY

1. THE JOURNAL OF PARASITOLOGY, as the official organ of the Society, shall be managed by an Editorial Committee appointed by the Council for a five-year period, and shall consist of one Helminthologist, one Protozoologist, and one Entomologist, one of whom shall be appointed by the Council to act as Chairman.

2. The Editorial Committee shall be assisted by an Editorial Board consisting of twelve members appointed by the Council for a four-year period in such a way that three members will retire and three new members shall be elected each year. The Editorial Board shall consist of six Helminthologists, four Protozoologists, and two Entomologists, to be elected on the basis of attainment, interest in the Society and geographical location.

3. The price of THE JOURNAL OF PARASITOLOGY shall be five dollars per volume, except to members of the American Society of Parasitologists who shall receive it as a membership privilege included in the annual dues of four dollars.

ADDITIONS AND AMENDMENTS

1. Additional By-Laws may be created by a two-thirds vote of Council members present at a meeting, or by an affirmative vote of nine Council members in a ballot conducted by mail. By the same procedure existing By-Laws may be repealed, amended, or suspended.